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IMMUNITY IN PREGNANCY

*A clinical study of the antistreptolysin,
antistaphylolysin and dye test reactions
and the gamma-globulin level*

BY

CLARENCE MALMNÄS

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FROM THE GYNAECOLOGIC CLINIC (HEAD: PROFESSOR H. SWANBERG),
THE SOUTH STOCKHOLM HOSPITAL, STOCKHOLM

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PREFACE

My interest in the influence of non-specific infections in pregnancy, especially in connection with premature birth and stillbirth, was instilled by Dr J. H. Magnusson, who was paediatric consultant at the Gynaecologic Clinic of the South Stockholm Hospital. He and Prof. S. Gard had planned an investigation of toxoplasmosis and pregnancy, which was begun about the same time as my own study, and in which the same case series was used. However, owing to the death of Dr Magnusson the toxoplasmosis study was not completed, and through the kindness of Professor Gard I took over the material, which was afterwards supplemented.

To Professor B. Lundqvist, then head of the Gynaecologic Clinic, I am greatly indebted for his encouragement and permission to use the material of the clinic.

During the course of the work I have had the pleasure of co-operating with specialists in various fields, who have given invaluable help. The investigation was planned and begun in co-operation with Dr Sven Löfgren and Dr C. A. Adamson, who had previously studied non-specific infection in relation to tuberculosis and other diseases. Complement fixation tests for influenza and parotitis were performed by Dr H. Lundbäck and for poliomyelitis by Dr A. Svedmyr. Through the kindness of N. A. Hellström it was possible to perform the biochemical studies on maternal and cord blood at the research laboratory of AB Kabi, in collaboration with H. Nihlén. The infra-red spectrum analysis for γ -globulin were carried out by Prof. E. Ingelstam and Dr L. Hultdt. The serologic work on various globulin fractions was performed by Dr G. Tunevall.

To Professors S. Gard, B. Malmgren, T. Packalén and H. Swanberg, Drs G. Hultdt, F. Wahlgren and P. Varenius and colleagues at the Clinic I would express my appreciation of their willingness to discuss problems arising during the investigation.

Valuable help has been received from the midwives and nurses at the Clinic, without whose co-operation this study would not have been possible.

Advice on statistical matters has been obtained from Dr G. Blom.

The research has been supported by grants from the City of Stockholm and the Swedish Medical Research Council.

To all who, in various ways, have made this investigation possible I tender my thanks.

CLARENCE MALMNÄS

The South Stockholm Hospital,

April, 1958

CONTENTS

INTRODUCTION	7
CHAPTER I Survey of the literature	9
CHAPTER II Case series	24
CHAPTER III Methods.	29
CHAPTER IV Antistreptolysin reaction	35
CHAPTER V Antistaphylolysin reaction	44
CHAPTER VI Bact. coli agglutination test	52
CHAPTER VII Dye and complement fixation tests.	55
CHAPTER VIII Transfer of antibodies across the placenta.	66
Serologic study	66
Biochemical study.	71
Electrophoretic examination of foetal serum.	71
Electrophoretic examination of maternal serum	74
Ultracentrifugal examination of foetal serum	74
Fractionation.	74
CHAPTER IX Serologic and ESR study of pathologic cases	80
Abortion	80
Still birth	82
Toxaemia of pregnancy	84
Toxoplasmosis	86
CHAPTER X General discussion and conclusions	89
CHAPTER XI Summary	94
REFERENCES	97

INTRODUCTION

"Immunity is one of the weapons in the basic struggle for life and denotes the resistance which an organism offers against aggression by a parasite" (Boyd,²⁸ 1956). The term immunity is usually employed not only in its literal sense of insusceptibility to infection, but generally to indicate various degrees of resistance; some authors who prefer the term resistance nevertheless use the word immunity (Topley & Wilson,²²⁴ 1955). It is usual to distinguish between innate and acquired immunity. Experimental studies on animals have revealed examples of inherited resistance to various infections, but in man this type has received little attention. Acquired immunity is generally divided into active and passive. An example of natural passive immunity is the congenital type. Immunity may be influenced by such factors as age, sex, hormonal status and nutrition. During the last decade the significance of the hormonal factor has been much studied. Whether pregnancy, which involves considerable hormonal changes, is accompanied by a variation in the resistance to infection has not been established, and the aim of this study was to throw light on this obscure problem. Several earlier studies in this field have been of the retrospective type. For instance, when a newborn has been found to have congenital toxoplasmosis, inquiries about the mother's health during pregnancy have often been made *afterwards*, as a result of which the course of the disease in the mother was judged to be normal. It is probable, however, that in such a case information on the mother's health *during* pregnancy would prove more valuable. This study was planned as of the prospective type. During the course of the study a number of problems calling for special consideration arose, some of which have been dealt with elsewhere.^{4, 134-137} This report is concerned chiefly with the following: (1) The antistreptolysin and antistaphylolysin reactions, *Bact. coli* agglutinins, and the Sabin-Feldman dye test during normal pregnancy. (2) The transference of antibodies from mother to foetus, studied by serologic and biochemical methods. (3) The use of the above serologic reactions and the erythrocyte sedimentation rate in some pathologic conditions during pregnancy.

CHAPTER I

SURVEY OF THE LITERATURE

That pregnancy is a biologic function that should for most women imply a period of happiness and satisfaction is almost a truism. However, for many—perhaps the majority—pregnancy involves some degree of stress, and such persons may well react to pregnancy as one might react to a disease.

Per Gustaf Cederschjöld,³⁸ an obstetrician to whom the development of the science of childbirth in Sweden owes much, wrote more than one hundred years ago: "The extent of the influence of pregnancy on the whole body is best seen in the blood, which then displays practically the same features as if there were an internal inflammation." Examples of these now recognized changes in the blood during pregnancy are leukocytosis,⁸ a leftward shift in the leukocyte picture⁸³ and a rise in the erythrocyte sedimentation rate^{70, 71} (Table I).

A marked increase in the histaminase in the blood is a normal reaction in pregnancy; it begins as early as the 2nd month and continues until the 6th, after which a rather constant level is maintained^{6, 194}.

It has been shown that this enzyme is produced in the decidua, which constitutes the boundary between the mother and the foetus.¹⁹⁴ It is generally believed that the histaminase protects the foetus from invasion by toxic diamines, but it has also been maintained that this enzyme may serve to protect the mother from toxic products from the foetus.¹⁹⁵

Endocrine role of the placenta

The above-mentioned changes in the blood during pregnancy are intimately connected with the hormones produced by the placenta. It was suggested at the beginning of the century that the placenta may be a gland of internal secretion⁸⁶ but the foundation for the conception of the placenta as an endocrine organ was laid in 1927, when Aschheim & Zondek discovered a gonadotrophic factor in the urine and serum of pregnant women. The placental source of this hormone was demonstrated in tissue culture of chorionic villi, where gonadotrophin was still recoverable in active culture after some months.⁴⁸ The chorionic gonadotrophin concentration of the human placenta has been found to reach a maximum between the 2nd and 3rd months and then to decline between the 3rd and 4th months. From the 5th month until term the con-

TABLE 1 The erythrocyte sedimentation rate during pregnancy and the puerperium. Review of previous work

Source	Method	No. of cases	ERYTHROCYTE SEDIMENTATION RATE*														
			Months pregnant								Weeks postpartum						
			III	IV	V	VI	VII	VIII	IX	X	1	2	3	4	5	6	7
Fähræus (1921)			24	21	29	33	41	47	45		41					20	
Sweden	Own	45	15-34	12-35	12-50	20-43	30-51	16-75	8-80		22-78					13-29	
Kühnel (1925)																	
Denmark	Gram	15	10	14	15	18	20	22	26	25	28	23	15	10		7	
Rasmussen (1934)																	
Norway	Westergren	248	13	25	20	35	32		41		37						
Griffin (1934)																	
U.S.A.	Weiss	150	12	12	17	20	24	29	31					11			
Bager (1936)																	
Sweden	Westergren	216								57	61						
Vogt (1941)			14	19	22	23			24		26					12	
U.S.A.	Cutler	25	12-16	17-21	21-24	17-26			22-26		21-30					2-22	
Perabo (1946)									39		35	31	19			15	
Switzerland	Rothpletz	194							17-61		8-62		1-37			2-28	
Obermer (1948)																	
England	Balachowsky	88	35	36	43	41	47	50	50	50		33				22	
Karlström (1949)																13	
Sweden	Westergren	168										25	19	14		0-31	
Hamilton (1953)			14	21	27	32	33	40	49	40		0-53	0-41	0-35		14	
England	Wintrobe	35	6-30	5-47	11-48	4-48	16-50	12-63	21-62	2-52							
Tyler & Roess (1957)			23			22		29				38					
U.S.A.	Wintrobe	30	0-43			0-45		0-48								2-47	

* Means and, in italics, ranges.

centration of the hormone is at a rather low constant level.⁴⁸ Adrenocorticotrophic hormone is probably also produced in the human placenta.¹¹

Large amounts of oestrogen are present in the human placenta, and careful estimations of this hormone have shown that the human placenta is concerned with the production of oestrogen, and that the foetal organism also plays an active role in the metabolism of oestrogen.⁴⁸

Clinical and experimental oophorectomy performed during pregnancy provided early evidence of the formation of progesterone outside the corpus luteum.¹⁰ Progesterone has been shown to be present in the human placenta at term,¹⁶² and as much as 2 mg per kg placenta has been isolated from human placental tissue.⁴⁸

The relationship between the mesencephalon, the hypophysis, and other endocrine organs has been the object of various experimental and clinical studies,^{183, 222} many of which have been concerned with ACTH and corticosteroids.

The hormonal relationship between the maternal and the placental and foetal adrenal functions in normal and toxæmic pregnancy has been widely studied.^{23, 25, 31, 46} The level of 17-hydroxycorticosteroids in the blood of pregnant women has been found to be very high in the last trimester, and especially after delivery; this is ascribed to stimulation of the adrenal cortex through stress experienced at this time.⁷⁵ At present there is little knowledge of the interactions between hormones from the placenta and maternal and foetal adrenal cortices. Tritium-labelled cholesterol has been found to traverse the human placenta in early and late pregnancy.⁴⁶ A high rate of cholesterol synthesis has been demonstrated in the foetal adrenals during the 3rd to 5th months of intra-uterine life, but in contrast to these findings there was no evident synthesis of cholesterol in the adrenals of an anencephalic infant.¹⁶⁷

These hormonal changes may constitute an important factor in resistance to infection in pregnancy, but knowledge in this field is scanty.

ANTIBODIES AND ANTIBODY PRODUCTION

There is general agreement that the serum antibodies are specialized globulins.^{36, 47, 161, 202} Most of the antibodies have an electrophoretic mobility similar to that of γ -globulin.^{155, 202} Some of them have been reported in the β -globulin fraction²⁸ and the Wassermann antibody has been said to have a mobility lying between β - and γ -globulin.^{45, 123}

Ultracentrifugal studies have revealed at least 3 components of γ -globulin with different molecular weights.^{28, 65} About 85–90 per cent of γ -globulin has a molecular weight of 160,000, the molecules being ellipsoids with a major axis

of about 350 Å and a minor axis of 40 Å; the rest consists of components with molecular weights of 300,000–1,000,000.

Studies of labelled proteins have shown that the plasma proteins, like cellular proteins, are in a state of flux and that the half-life of antibody globulins is about 2 weeks.¹⁸¹

The normal range of the γ -globulin level in adults is between 700 and 1100 mg per 100 ml serum.⁷⁶ Since 1952 an increasing number of cases of agammaglobulinaemia have been reported—that is, patients showing less than 100 mg γ -globulin per 100 ml serum. In the clinical history of these cases there have been severe recurrent infections.^{32, 77, 138} At least 2 types of this disease have been described—a congenital sex-linked recessive form occurring in males, and an acquired form occurring in both sexes.

Hypogammaglobulinaemia has been reported in endocrinal disturbances, especially where there is overproduction of cortisone.²⁸

Many theories on the mechanism of antibody formation have been advanced, but none of them has become generally accepted.

The site of antibody production

Metchnikoff⁴⁹ advanced the view that the antibodies were a secretion from the macrophages, which phagocytized and destroyed the cells containing antigen. Such cells adapted to deal with foreign material by phagocytosis are found principally in the liver, spleen, bone-marrow and lymph nodes, and constitute what Aschoff called the reticulo-endothelial system. The reticulo-endothelial theory of antibody production, which has gained wide acceptance, finds support in the fact that intravenously injected India ink and certain dyes such as trypan blue collect mainly in the reticulo-endothelial cells. Blockage of this system with, for instance, colloidal iron results in a decrease in the antibody production. Some decades ago it was suggested that lymphocytes were the producers and transporters of antibodies⁵¹—a view based partly on the finding that an extract from the lymphoid cells of an immunized mouse contained twice as many antibodies as the blood serum,⁴⁹ and that irradiation which produced lesions in lymphoid tissue effected a reduction in the antibody production.

In foetal lymph glands reaction centres do not appear until after birth.⁴⁹ It has been suggested that plasma cells are probably the most important producers of antibodies and serum globulins. A relationship between hyperglobulinaemia and these cells has been suggested.^{26, 51} The highest level of gamma globulin has been found in myeloma, whereas there was no similar increase in the globulin fraction in lymphatic leukaemia. A marked increase in globulin and massive plasma cell proliferation in the spleen on immunization

of the rabbit with polyvalent pneumococcus vaccine suggests that the plasma cells form gamma globulin.⁵⁶

In a study by Caspersson's method it was found that plasma cells presented much the same cytochemical picture as is found in protein-forming cells, with marked deposition of nucleic acid in the cytoplasm.²⁴ In tissue cultures of the spleen from the immunized rabbit it was discovered that red pulp containing many plasma cells formed greater quantities of antibodies than white pulp consisting chiefly of lymph follicles. From the same study it was concluded that the plasma cells derive from reticulum cells and that the development is represented by the following morphologic chain: reticulum cell-transitional cell-immature plasma cell-mature plasma cell. It was inferred that antibodies are produced by reticulum cells and that the production of antibodies corresponds to the differentiation of reticulum cells to plasma cells. The maximum of the titre curve corresponded to the early stage of plasma cell development.⁵⁶

In studies of the response to various antigens it was found that the rise in antistreptolysin and antistaphylolysin titres often occurred at the same time as, or shortly after, the central reaction in the spleen.¹⁷²

Local production of antibodies

Studies on cattle indicate that antibodies are formed in the walls of the uterus and vagina. In these studies *Trichomonas foetus* and *Brucella abortus* were used as antigens. When alum-precipitated tetanus toxoid was introduced into the cavity of the uterus and vagina of primarily stimulated rabbits, a local production of tetanus antitoxin occurred in the walls of these structures.¹⁸

Hormones and antibody production

ACTH and cortisone have gained wide clinical application in the last decade. An undesirable effect, however, is not uncommon, the resistance to infection being reduced; latent infections have even been known to flare up during treatment with corticosteroids. In experimental studies, especially on rodents, a probable inhibition of the synthesis of antibodies by cortisone has been demonstrated, although it does not appear to act on passively supplied antibodies.²⁰

Burnet & Fenner³⁶ have summarized their views on antibody production as follows: (1) Antibody production against antigens that have entered a local tissue occurs principally in the regional lymphatics and then mainly in plasma cells. (2) The spleen plays a similar role when the antigen is in the blood. (3) The liberation of antibodies from plasma cells (and lymphocytes) probably occurs under control of corticosteroids.

Antibodies in pregnancy

Most of the present knowledge on antibodies and antibody formation has been obtained by experimental studies on animals. In view of the difficulties of applying conclusions drawn from work on animals to humans, the literature concerning antibodies in pregnancy is reviewed in two sections: (A) Animal studies and (B) Human studies.

ANIMAL STUDIES

Serologic methods

The congenital immunity was studied by Ehrlich⁵² in 1892. By feeding mice on ricin and abrin he developed in the animals a strong immunity to these plant toxins. When the father, but not the mother, was immunized against these toxins, no immunity was created in the offspring, from which it was concluded that the idioplasm of the spermatozoon is unable to transmit immunity. As regards the importance of the mother to congenital immunity, Ehrlich considered that it was necessary to use animals which had been immunized before fecundation. If the immunization tests were performed during pregnancy, an active immunization of the foetus could not be excluded. In his tests Ehrlich found that in all cases where the female animal was immunized a marked immunity could be demonstrated in the young. Six weeks after birth the immunity had diminished appreciably in the young, and after three months it had disappeared completely. Ehrlich considered that the immunity was of the passive type in view of the transfer of antibodies across the placenta. In contrast to Ehrlich, however, von Behring¹⁹ considered that the placenta functioned as a dialyzer through which the antitoxins passed only with difficulty owing to their molecular size.

Wegelius²¹⁵ developed active immunity in the goat and rabbit both before and during pregnancy by means of vibriolysin, tetanus toxin and *Bact. coli*. The antibodies to these antigens could be demonstrated in the blood of the young on birth. In the rabbit the titre value was the same for mother and foetus at delivery, whereas in the goat the titre was higher for the young. From his studies Wegelius drew the conclusion "dass wenn Serum eines schwangeren Tier Antikörper enthält dieselben auch bei den Jungen wiederzufinden sind. Es erscheint mir aus meinen Versuchen hervorzugehen, dass die Übertragung von Antikörpern von Mutter auf Kind nicht als ein einfacher Filtrationsprozess aufzufassen ist, sondern dass die Placenta eine elektive Kraft gegenüber den Stoffen, welche im mütterlichen Serum vorkommen, zuerkannt werden muss".

The absence of agglutinins against *Bact. coli* and *Br. abortus* in the newborn calf even when the mother displayed a high titre has been regarded as evidence that the cow placenta is impermeable to these antibodies.^{126, 187} It was found

TABLE 2 Schematic survey of placentation in mammalian species
(after Grosser and Mossman)

Tissues composing the separation between maternal and foetal blood									
Morphologic type	Maternal tissue				Foetal tissue			Relation to endometrium	Typical examples
	Endo- the- lium	Con- nec- tive tissue	Epi- the- lium	Uterine lumen	Troph- oblast	Con- nec- tive tissue	Endo- the- lium		
<i>Semiplacenta</i>									
Epithelio-chorial	+	+	+	+	+	+	+	Non-deciduate	Pig, horse
Syndesmo-chorial	+	+	—	+	+	+	+	Transitional	Sheep, goat, cow
<i>Placenta vera</i>									
Endothelio-chorial (vasochorial)	+	—	—	—	+	+	+	Deciduate	Cat, dog
Haemochorial	—	—	—	—	+	+	+		Man, monkey
Haemo-endothelial	—	—	—	—	—	—	+		Guinea pig, rat, rabbit

that the agglutinin content rose after supply of colostrum, but that this had to be given not more than 18 hours after the birth of the calf for there to be any resorption of the antibodies from the colostrum through the intestinal mucosa.

In a series of experiments on horses, pigs, cows and sheep which were actively immunized against typhus and diphtheria, it was found that the antibodies were transmitted chiefly by colostrum to the newborn. In the dog, antibodies passed through the placenta only in small quantities, most of them reaching the pup via the colostrum, while in the rabbit the antibody content was about the same in the newborn young prior to suckling as in the doe. It was concluded that the transfer from mother to foetus depended on the type of placenta according to Grosser's scheme^{179, 180} (Table 2).

Using *Clostridium welchi* alpha toxin and *Brucella abortus* as antigens, and sera prepared in man, the rabbit, guinea pig, dog, cow and horse it was found that antibodies did not pass the rabbit placenta as had previously been supposed, but the transfer from mother to young occurred by way of the uterine lumen and the foetal yolk sac splanchnopleur of the area vasculosa.³⁰ The route in other species, including man, was not established.

It has been shown that protection against mouse encephalomyelitis virus

can be passively transmitted from the immune female mouse to the foetus, and that the inoculation of the young of an immune mother with an active virus develops an active immunity. Immunity was also produced by oral ingestion of live virus during the decline in congenital passive immunity.⁴³

Biochemical methods

The relationship between the plasma proteins and immunity has been studied chiefly by salting out and by electrophoresis. With the former method neither euglobulin nor pseudoglobulin were detected in the serum of the newborn calf until colostrum had been supplied.¹⁰¹

Studies in the electrophoretic mobility of serum proteins of newborn calves, foals, lambs and pigs have shown that whereas no measurable γ -globulin could be found at birth it was present in the serum a few hours after the animals had received colostrum^{109, 143, 169} (Table 3).

Pedersen¹⁶³ found a hitherto undescribed globulin component—between α_2 and β ,—in the serum from foetal and newborn ruminants. He called the component fetuin and gave its molecular weight as about 50,000. In serum from 8–9-month calf foetus, fetuin constituted 33 per cent of the total serum protein and about 90 per cent of the globulin fraction. For newborn calves the corresponding figures were 20 and 50–80 per cent, and in 6-month-old calves 5 and 20 per cent. He also found fetuin in the lamb, but only in small quantities in rabbit foetus. The physiologic importance of this globulin component is unknown, though Pedersen noted that the content was high in ruminants, which have a syndesmochorial placenta, but low in man with a haemochorial placenta (Table 2).

An electrophoretic study revealed no γ -globulin in foetal serum of sheep at 98 and 112 days of pregnancy.¹⁴³

STUDIES IN THE HUMAN SUBJECT

Serologic methods

At the turn of the century a number of papers were published on antibodies in the mother and foetus, attention being centred on the possible transfer of *agglutinins* across the placenta in cases of typhoid fever. Agglutinins against typhoid bacillus were sometimes found in the newborn and at a lower titre than in the mother.^{2, 110} It was later found that typhoid H agglutinin passed the placenta in a considerable number of cases, whereas the O agglutinin was scarcely transmitted at all.²⁰¹ In a series of 40 women vaccinated against typhoid fever during pregnancy a normal output of agglutinins was evident. Only in exceptional cases were agglutinins against typhoid bacillus found in the cord blood.⁷³

Earlier investigations suggest that agglutinins against *Bact. coli* are able to

TABLE 3 Variation of protein composition of serum with age in the cow, horse and sheep

The figures are percentages

Source	Age of the animal	Albumin	Globulins		
			α	β	γ
Jameson <i>et al.</i> , 1942	<i>Cow</i>				
	Newborn	57	37	6	—
	18 hrs	50	35	9	6
	36 hrs	30	22	7	41
	2 years	40	10	6	44
Polson, 1943	<i>Horse</i>				
	Newborn	65	32	3	—
	5 days	37	36	25	2
	8 months	33	14	25	28
	10 years	30	11	12	47
Moore & McCarthy, 1946	<i>Sheep</i>				
	Foetus 98 days	65	35	—	—
	Mother 98 days	44	50	—	6
	Foetus 112 days	65	35	—	—
	Mother 112 days	38	48	—	14

pass the human placenta, but that there was a tendency for them to be filtered off.^{117, 203, 216} However, later studies have shown that these agglutinins do not generally occur in the cord blood, though the mother may present a fairly high titre.³

The *antistreptolysin* and *antistaphylolysin* titres at term have been found to be at about the same level, or slightly higher in the newborn than in the mother.^{3, 33, 37, 212} Blood samples taken from cord and maternal blood at various stages of pregnancy showed a rising foetal level of these antibodies during the second half of pregnancy.^{135, 211}

In a study of antibodies against *H. influenzae* by the *complement fixation test* it was found that the antibody level was appreciably higher in maternal than cord blood.²⁰⁶

The levels of diphtheria and tetanus *antitoxins* in maternal and cord blood were approximately equal.^{125, 210} Immunization of 27 pregnant women in the last trimester with diphtheria toxoid in order to protect the offspring generally resulted in a moderate rise in the antitoxin level of the maternal blood; at delivery the titre values in both bloods were the same.²¹⁰

Biochemical methods

It appears to be generally agreed that there is a decrease in the total protein value during pregnancy. Electrophoretic studies using Tiselius' technique

TABLE 4 Electrophoretic analysis of serum proteins in normal pregnancy and postpartum

The figures are percentages

Source	Method ^a	Stage of pregnancy	No. of cases	Albumin	Globulins			
					α_1	α_2	β	γ
Lagercrantz ^b (1945)	*	3rd trimester	12	45.5	12.2		21.9	20.9
Longworth <i>et al.</i> (1945)	*	One hour post-partum	11	49.7	6.7	11.0	22.6	10.0
Neuweiler (1948)	*	2nd trimester	3	46.0	15.7		20.6	17.7
Moore <i>et al.</i> (1949)	**	3rd trimester	7	54.7	12.8		19.5	13.0
Ewerbeck & Levens (1950)	****	Term	28	51.7	14.4		18.7	15.2
Malmnäs & Nihlén (1951)	*	Retroplacental serum	100	44	8	12	22	14
Brown (1954)	***	1st trimester	10	59.0	7.5	8.5	13.0	12.0
		2nd trimester	10	55.0	8.0	11.0	15.0	11.0
		3rd trimester	10	44.5	9.1	15.9	20.3	10.2
Brown (1956)	***	Prepartum	8	41	7	18	20	14
		Postpartum	8	38	6	16	22	18
MacGillivray & Tovey (1957)	***	1st trimester	25	57.5	4.0	8.9	13.8	15.8
		2nd trimester	25	50.6	5.3	11.8	18.3	13.9
		3rd trimester	25	50.6	5.9	11.7	18.9	12.9
Lagercrantz (1945)	*	Nonpregnant	16	59.4	6.1		14.4	20.1
Brown (1954)	***	Nonpregnant	5	59	5	10	14	12
MacGillivray & Tovey (1957)	***	Nonpregnant	100	63.4	3.3	7.1	12.7	13.5

^a * = Tiselius' apparatus; ** = Tiselius' apparatus (microcell); *** = Paper electrophoresis; **** = Antweiler apparatus (microcell).

^b Lagercrantz: phosphate buffer pH 7.7. Others: veronal buffer pH 8.6.

and/or paper electrophoresis have shown that there is a significant decrease in the albumin component and a corresponding increase in β -globulin, which consists largely of lipo- and glyco-proteins.

Some studies suggest a decrease in the γ -globulin fraction, and a slight increase in the α_1 and α_2 fractions.

A survey of the literature concerning electrophoretic analysis of the various serum protein fractions during pregnancy and postpartum is summarized in Table 4. The variations between the results found by the quoted investigators are probably due to the difference in the techniques.

TOXOPLASMOSIS

Toxoplasmosis is a world wide infectious disease caused by a protozoon, *Toxoplasma gondii*. As early as 1908 Nicolle & Manceaux found *Toxoplasma* in a rodent, *Ctenodactylus gondii*, which was being used in experiments at the Pasteur Institute in Tunis. The same year Splendore found the parasite in the rabbit in Brazil. It is now known that *Toxoplasma* appears in various types of animals, and principally in mammals and birds.²⁰⁰ In several studies toxoplasmosis has been found in dead hares.^{40, 103} The disease has also been recorded in common domestic animals such as dogs and cats.¹¹⁹

That *Toxoplasma* is pathogenic for man was not known until 1939 when the protozoon was isolated from an infant who had died from a congenital infection.²²⁷ Some years later *Toxoplasma* was found in adults who had died after severe infections with high temperature, exanthema and atypical lung changes.¹⁶⁶ There have been several reports, especially from Scandinavia, of a relatively common mild form of acquired toxoplasmosis characterized by enlargement of the lymph glands, with or without fever, and frequently presenting a mononucleotic blood picture.^{4, 72, 185, 186} A case of particular interest in this connection has been recorded by Gard & Magnusson⁷² and will be described here.

A 23-year-old pregnant woman, who, like the husband, had always been healthy, had her last normal period on 6th June, 1949, and fell ill with low-grade fever (maximum 38.2) on 24th. After some days she experienced aching in the neck and throat. Some weeks later the temperature had subsided and she felt well again, except that the aching remained. An examination on 1st July showed her general condition to be satisfactory, with a temperature of 37.3. In the neck and at the right angle of the jaw two bean-sized, mobile, slightly aching lymph nodes were palpated. The oral cavity and throat were normal, as were the internal organs. The erythrocyte sedimentation rate was 27 mm in one hour. Lung radiographs were noncontributory. On examination a week later similar swellings were observed in the axillae and in the groins. Histologic examination of the neck nodes revealed an inflammatory reaction in the lymphatic tissue that was suggestive of toxoplasmosis. A follow-up on 22nd August showed the lymph nodes to have diminished considerably in size, and the patient felt well.

On 27th September she attended the maternity centre of the South Stockholm Hospital where normal pregnancy was established. The routine check performed with particular attention to the possibility of toxoplasmosis included a dye test (Sabin-Feldman); a titre value of 1:50 was found, which in the course of 2 months rose to 1:2000. In detail, the titres were: 22nd November 1:2000, 12th December 1:2000, 30th December 1:4000, 13th January 1:1000, 19th February 1:500.

The pregnancy followed a normal course and on 16th March, 1950, the patient gave birth to a girl weighing 3500 Gm. There were no signs of congenital toxoplasmosis, and this was confirmed at subsequent examination.

Two similar cases reported in the literature however presented foetal infection, with signs of congenital toxoplasmosis in the newborn.¹⁸⁶

In a recently reported series of acquired toxoplasmosis 12 out of 13 cases were women, and of these one became pregnant one month after the clinical debut of the disease.⁴ During pregnancy, which took a normal course, the dye test titre rose to 1:4000. The child weighed 3760 Gm at birth and presented no signs of toxoplasmosis.

Another recently published study¹⁰² on toxoplasmosis includes a case of high dye test titre during pregnancy.

A clinically asymptomatic 26-year-old gravida V in the 9th month of gestation had had four still births. Blood tests revealed a dye test titre of 1:1000 and complement fixation test titre of 1:120. She gave birth to a healthy child at term. There was no sign of toxoplasmosis at subsequent clinical and serologic examinations.

Congenital toxoplasmosis, which is generally characterized by hydrocephalus, cerebral calcifications, chorioretinitis, convulsions and mental backwardness, is generally presumed to originate from the mother who contracted the infection during pregnancy, the disease often taking a subclinical course.^{15, 57, 60, 68, 74} On the other hand it has been maintained that the congenital infection has its origin in a chronic infection of the mother which flares up during pregnancy. Whether it is a question of an acute toxoplasmosis or an acute transformation of a chronic infection, the foetus is probably infected through the entry of the *Toxoplasma* into the placenta in parasitaemia, after which it penetrates from cell to cell or through defects in the placenta. In a recent study¹⁰² of 8 cases of congenital toxoplasmosis, 5 of which had hydrocephalus, 7 plexus calcification, 7 chorioretinitis and/or microphthalmia, 2 purpura and 2 icterus, three of the children died of the disease at ages of 5 months, 6 months and 2½ years.¹⁰²

PLACENTAL TRANSMISSION OF VIRUSES

Since it has been demonstrated that viruses grow rapidly in embryonic tissue and that there is a connection between virus infection during the first trimester of pregnancy and congenital defects, research in this field has been intensified.

Small-pox. In the days of small-pox the disease was seldom seen in pregnant women, but when it did occur the mortality rate was greater than in non-pregnant subjects.⁸¹ The foetal mortality was about 30–50 per cent. Intra-uterine infection was said to be common and occasionally the newborn child had pock marks—as for example, the famous French obstetrician Mauriceau.

Chicken-pox in pregnancy is rare but there are reports that intra-uterine transmission of this virus does occur.⁸¹

Measles during pregnancy was formerly regarded as a serious disease. In a

TABLE 5 Incidence of abortion, still birth and congenital malformation in relation to some virus infections during pregnancy

	Infection during first three months of pregnancy				Infection after first three months of pregnancy			
	Abortion or still birth	Live birth with malformation	Normal newborn	Total	Abortion or still birth	Live birth with malformation	Normal newborn	Total
Rubella	13	16	60	89	4	5	67	76
Morbilli	1	7	54	62	—	3	32	35
Parotitis epidem.	5	7	39	51	3	6	62	71
Varicella	—	—	25	25	3	1	32	36
Poliomyelitis	31	3	30	64	30	5	143	178
Total	50	33	208	291	40	20	336	396
Per cent	17.2	11.3	71.5		10.1	5.1	84.8	

series¹⁵³ of 84 cases the maternal mortality was 14 per cent, and the foetal mortality about 50 per cent. In another series⁵⁴ of 52 cases, 23 of the pregnancies ended in abortion or premature birth. Antibodies against measles are presumed to pass through the placenta and the newborn usually has a passive immunity to the disease (Table 5).

Mumps. The incidence of epidemic parotitis in pregnancy is rare. In a large investigation in Sweden,⁸⁴ where mothers at delivery were asked if they had had any virus disease during pregnancy, 30 out of 24,549 stated that they had had mumps.

Epidemic hepatitis. The incidence of hepatitis is lower in pregnancy than in non-pregnant women.¹⁹⁶ In the above-mentioned study⁸⁴ 21 out of 14,926 patients questioned (0.14 per cent) had had infectious hepatitis during pregnancy. Among these 21 cases there were 2 abortions, 4 premature births and one child with palatoschisis.

Influenza. Pregnant women are said to be particularly susceptible to influenza.⁸¹ In a large series⁹¹ of 1350 pregnant women with influenza in the United States during the 1918 epidemic, about one half of the cases developed pneumonia, and among them the mortality rate was about 50 per cent. The total mortality rate was 27 per cent, and the foetal mortality varied between 20 and 50 per cent in various groups.

Rubella. Since Gregg (1941) reported that rubella in pregnancy can cause malformation of the child an extensive literature has accumulated on this subject. Some of the results published are summarized in Table 5. During the first half of 1941 Gregg, as an ophthalmologist, saw 13 children with various defects of the eyes, mostly cases of congenital cataract. When he made retrospective inquiries he found that the mothers had had rubella during the first

months of pregnancy. In the summer months of 1940 there had been a large epidemic of rubella in Australia.

This epochal observation has since been confirmed from all parts of the world, and it is now established that maternal rubella during the first trimester of pregnancy can cause certain types of congenital defects of the child, especially cataracts, heart lesions, deaf-mutism and microcephaly.¹³⁰

The literature^{1, 50, 84, 127, 130} on the influence on the foetus of maternal rubella in pregnancy has been summarized in Table 5. There were 89 cases where the mother had rubella in the first trimester of pregnancy, and in this group there were 13 abortions and still births, 16 defective live-born children and 60 with no defects. There were 76 women who contracted rubella after the first trimester, and here there were 4 abortions and still births, 5 defective live-born children and 67 children with no defects. Comparison of the frequency of abortions and still births in both groups and a similar comparison of the number of live-born children with defects revealed no significant differences.

Poliomyelitis

Pregnancy is said to render a person susceptible to poliomyelitis. In one study,²¹⁴ however, it was held that there is greater immunity to poliomyelitis in the first trimester, which may be ascribed to the large amount of chorion-gonadotrophin produced in the placenta.

Other workers,^{29, 208} who found 586 cases reported in the literature, were unable to confirm this opinion, but found that the distribution according to trimester of gestation was uniform.

Among 428 cases of poliomyelitis in pregnancy²⁹ there was a death rate of 9.7 per cent, which was significantly higher than the figure for a non-pregnant group. More than one half of the maternal deaths occurred in the last trimester and in puerperium. It was formerly thought that the polio virus did not pass the placental barrier, but more recently there have been reports of intra-uterine infection of the foetus.

Antibodies against polio on the other hand are said to pass from mother to foetus. In a study²¹⁸ of 51 cases of poliomyelitis in pregnancy there were 14 in the first, 20 in the second and 17 in the third trimester. There were 6 maternal deaths, 7 abortions and 3 still births. The incidence of abortion, still births and newborn with malformations seemed higher when the virus infection occurred during the first trimester. The differences are not significant, however, owing in part to the small size of the groups.

CONCLUSIONS

For all virus infections together there was a higher percentage of both abortions (including still births) and newborn with malformations when the infection occurred during the first trimester, the difference being significant (Table 5).

CHAPTER II

CASE SERIES

The case series for this investigation was selected from patients attending the Gynaecologic Clinic of the South Stockholm Hospital who expressed willingness to attend for regular examinations during the course of pregnancy and whose veins were in good enough condition to permit blood samples to be drawn without difficulty. The major part of the series was collected from 1949 to 1953, after which date it was supplemented by a number of pathologic cases.

NORMAL GROUP

The normal group comprised cases satisfying the following conditions:

- (1) No history of serious infections, such as active tuberculosis, syphilis and haemorrhagic nephritis.
- (2) No history of non-specific infections—for instance of the throat, respiratory tract and intestinal or urinary tract—during the 3 to 4 months preceding pregnancy.
- (3) No manifest morbid conditions during pregnancy, such as infections, haemorrhage, obscure pains, anaemia (haemoglobin less than 70 per cent) and toxæmia of pregnancy.

High antibody titres and/or high erythrocyte sedimentation rate did not exclude the patient from the normal group if there were no signs of disease.

- (4) Delivery at full term of a living child, weighing over 2500 Gm. Gravidæ associated with multiple births were excluded from the normal group.

The antibody titre tests were performed in the case of 1887 gravidæ satisfying requirements (1) and (2). Of these, 232 discontinued attendance on such grounds as change of address, miscarriage or apprehensions concerning blood sampling.

Requirements (3) and (4) were not fulfilled by 538 subjects. The remaining 1117 cases constituted the normal group, there being 502 primigravidæ and 615 multigravidæ.

Table 6 provides a survey of the group with regard to age, parity, marital status and weight of child.

TABLE 6 Composition of the normal group

Birth weight (Gm)	Maternal age	Primigravidae		Multigravidae		Total
		Married	Single	Married	Single	
2501-3000	< 20	4	6	3	—	13
	20-24	16	5	19	4	44
	25-29	17	3	20	—	40
	30-34	10	3	20	2	35
	35-39	2	1	15	—	18
	≥ 40	1	—	6	2	9
3001-3500	< 20	17	14	4	1	36
	20-24	61	34	41	5	141
	25-29	62	6	52	8	128
	30-34	22	2	44	4	72
	35-39	5	1	28	2	36
	≥ 40	3	3	9	—	15
3501-4000	< 20	6	16	2	1	25
	20-24	61	15	42	7	125
	25-29	38	—	58	1	97
	30-34	20	4	59	3	86
	35-39	7	2	32	—	41
	≥ 40	1	—	14	1	16
> 4000	< 20	2	2	2	—	6
	20-24	14	2	10	4	30
	25-29	6	1	33	—	40
	30-34	4	1	32	2	39
	35-39	2	—	14	3	19
	≥ 40	—	—	5	1	6
Total		381	121	564	51	1117

CONTROL GROUP

To ascertain whether the selection of the case series for the normal group at the maternity centre was representative, comparison was made with a randomly drawn series from some 40,000 gravidae at the clinic during the period 1943-1953. The control group was that used in a former study of premature and still births. The subjects accepted for the group were those with a record number following that of a gravida giving birth before full term or being presented with a stillborn child, and on condition that the pregnancy resulted in a single birth of a living child weighing more than 2500 Gm.

Table 7 provides a survey of the control group.

TABLE 7 Composition of the control group

Birth weight (Gm)	Maternal age	Primigravidae		Multigravidae		Total
		Married	Single	Married	Single	
2501-3000	< 20	5	12	1	—	18
	20-24	30	15	18	2	65
	25-29	34	2	32	3	71
	30-34	12	1	40	1	54
	35-39	5	—	22	1	28
	≥ 40	—	—	4	1	5
3001-3500	< 20	13	17	5	2	37
	20-24	89	16	87	9	201
	25-29	86	17	139	12	254
	30-34	46	1	103	5	155
	35-39	17	5	42	4	68
	≥ 40	2	—	19	—	21
3501-4000	< 20	13	12	5	2	32
	20-24	85	12	69	12	178
	25-29	72	2	157	5	236
	30-34	38	1	137	2	178
	35-39	18	—	76	3	97
	≥ 40	1	—	16	2	19
> 4000	< 20	7	7	—	1	15
	20-24	28	3	22	2	55
	25-29	32	3	76	4	115
	30-34	9	1	65	2	77
	35-39	5	—	44	1	50
	≥ 40	—	—	9	—	9
Total		647	127	1188	76	2038

COMPARISON BETWEEN NORMAL AND CONTROL GROUPS

BIRTH WEIGHT

The birth weights were about the same in normal and control groups, with a tendency to greater values in the latter group (Table 8).

MATERNAL AGE

The normal group contained more subjects under 25 years of age, the difference being significant***. The 25-29 year group, on the other hand, was better represented among the controls. The ≥ 30 year group was equally represented (Table 9).

TABLE 8 Birth weights in normal and control groups

Birth weight (Gm)	Normal group		Control group		Difference (%)
	No.	%	No.	%	
2501-3000	159	14.2	241	11.8	2.4*
3001-3500	428	38.3	736	36.1	2.2 ^o
3501-4000	390	34.9	740	36.3	1.3 ^o
> 4000	140	12.5	321	15.8	3.3*

TABLE 9 Age distribution in normal and control groups

Maternal age	Normal group		Control group		Difference (%)
	No.	%	No.	%	
≤ 24	420	37.6	601	29.5	8.1***
25-29	305	27.3	676	33.2	5.9***
30-34	232	20.7	464	22.8	2.0
≥ 35	160	14.3	297	14.5	0.2

MARITAL STATUS

The number of unmarried women was greater in the normal than in the control group*** (Table 10).

TABLE 10 Marital status in normal and control groups

	Single and married	Single only		Difference (%)
		No.	%	
Normal group	1117	172	15.4	5.4***
Control group	2038	203	10.0	

PARITY

Primigravidae were more numerous in the normal group*** (Table 11). Multigravidae previously having only abortions were more common in the normal group***. Subjects who had previously given birth to only one child were equally represented in both groups, and those with two children were more numerous in the control group, the difference being small in both cases compared with that for subjects who had previously given birth to three or four children (Table 12).

TABLE 11 Primigravidae in normal and control groups

	Total	Primigravidae		Difference (%)
		No.	%	
Normal group	1117	502	45	7***
Control group	2038	774	38	

TABLE 12 Previous pregnancies in normal and control groups

Previous pregnancy	Normal group				Control group				Difference (%)
	Married	Single	Total	%	Married	Single	Total	%	
Abortion (no child)	67	11	78	12.7	66	4	70	5.5	7.2***
One child	337	27	364	59.2	701	46	747	59.1	0.1 ^o
Two children	109	10	119	19.3	303	14	317	25.1	5.8**
Three or more children	51	3	54	8.8	118	12	130	10.3	1.5 ^o
Total	564	51	615		1188	76	1264		

DISCUSSION

The method of selection provided a fairly representative case series of clinically healthy gravidae. The tendency towards a lower age in the normal group was due to the preponderance of primigravidae, which is ascribable to the greater readiness with which mothers without children attended the frequent examinations. The greater number of single and divorced subjects in the normal group may have been due to the presence of a medical social worker at the maternity centre of the clinic, but not at the independent centres in the district. Gravidae at the latter centres requiring such assistance are not infrequently remitted to the clinic centre, where they subsequently attend.

It would have been preferable if a group from an independent maternity centre had been included in the study, but this proved impracticable. Neither was it possible to follow a large group of non-pregnant women of similar age over a period of 9-10 months, though this might well have been rewarding.

CHAPTER III

METHODS

On her first visit to the maternity centre of the clinic each gravida of the principal series was questioned on past infections and other diseases. In addition to the usual gynaecologic examination a physical check was made of the teeth, throat, heart and lungs, and the height and weight were noted. Blood samples were taken by venipuncture for WR, Rh, ESR and the anti-streptolysin and antistaphylolysin reactions. The haemoglobin determinations were performed on the capillary blood. All cases underwent fluoroscopic examination of the lungs.

Subsequent follow-ups were performed once a month, on which occasions information concerning any infections and symptoms was requested. The routine check of pregnancy was performed and samples taken for serologic, ESR and haemoglobin examinations. The urine was examined regularly for the presence of albumin, and in cases of suspected infection samples were taken for sedimentation tests; samples for cultures were sent to the bacteriologic laboratory. Where infection of the upper respiratory tract was suspected, samples were taken from the nose and throat for bacteriologic study. From patients with vaginal discharge samples were taken from the cervix for cultivation. Some one hundred cases were followed by cultures from the vaginal and cervical samples during various periods of pregnancy and where there was no sign of discharge*.

On a part of the series dye and complement fixation tests and the *Bact. coli* agglutination test were performed over a period. At delivery blood samples were taken from a small part of the series for complement fixation tests against parotitis, influenza and polio virus.

On groups of the series blood samples were taken for electrophoresis and fractionation by Cohn's method. The isolated γ -globulin was studied by infrared spectrophotometry.

At delivery, blood samples were taken from the mother by venipuncture, and from the umbilical cord before the pulsations in it had ceased. Precautions were taken to ensure that there was no intermingling of maternal and cord blood.

* The results are to be published later.

SEROLOGIC EXAMINATION*

The antistreptolysin test was performed according to the directions given by Kalbak,¹¹⁴ and Todd's standard serum was used.

Determinations of the antistaphylolysin titre were carried out by the technique due to Ipsen¹⁰⁴ and modified by Packalén & Bergqvist.¹⁵⁹

The *Bact. coli* agglutination test was performed by Vahlne's technique, both O and H antigens from about 40 strains being used.²⁰⁹ The routine dilution was 1:40 and after the addition of equal parts of antigen the value for the first test was 1:80. In 56 instances of maternal and cord serum the routine dilution was set at 1:10, so that the dilution for the first test was 1:20.

The complement fixation tests for parotitis and influenza were performed by the technique described by Lundbäck,¹²⁹ and for poliomyelitis by the Fulton's plate technique as modified by Svedmyr, Enders & Holloway.¹⁹⁷

For the determination of *Toxoplasma* antibodies used in this study the Sabin & Feldman dye test and the complement fixation test were performed.

The *dye test*. The technique described by Sabin & Feldman with a few modifications reported by Huldt¹⁰² was used. The dye test is based on the fact that the cytoplasm in *Toxoplasma* cells is methylenophilous. Addition of specific antibodies and an accessory thermolabile factor obtained from normal human serum inhibits the uptake of methylene blue by *Toxoplasma* cells. The dye test titre was stated as the highest serum dilution at which the unstained *Toxoplasma* cells amounted to at least 50 of 100 organisms counted.

The *complement fixation test* was performed according to the method of Warren & Russ,¹⁰² using chorioallantoic antigen. The titres were expressed as the serum dilution in reactive mixture.

ELECTROPHORESIS

The electrophoretic examinations of the sera of foetus of different ages were performed in a Tiselius apparatus (LKB products series no. 3023). A phosphate buffer of pH 7.6 and ionic strength 0.1 was used. With this apparatus it was not possible to separate the α_1 - and α_2 -globulins. An improved Tiselius-Svensson apparatus (LKB products series no. 3204) with a higher resolving power was employed later. A sodium veronal buffer of pH 8.6 and ionic

* The antistreptolysin and antistaphylolysin reactions and the complement fixation test against poliomyelitis were performed at the Central Bacteriologic Laboratory of Stockholm. The *Bact. coli* agglutination tests were done at the Department of Hygiene, the Caroline Institute. The Sabin-Feldman dye test and the complement fixation tests for parotitis and influenza were performed at the National Bacteriologic Laboratory.

strength 0.1 was used. Under identical conditions—buffer, protein concentration, electric potential gradient and electrophoresis time—the duplicate values for the concentrations of the various components differed by one per cent or less.

FRACTIONATION METHOD

Blood samples were taken at delivery from the umbilical cord and from the mother by venipuncture. The blood was collected in centrifuge bottles and allowed to clot. Blood corpuscles and fibrin were separated in an MSE refrigerated centrifuge. The sera were deep frozen at -25°C and stored until fractionation could take place.

The fractionations were performed by Cohn's method 6, in which the various protein fractions of the serum are precipitated with alcohol at low temperature in order to avoid denaturation. The pH values and ionic strength in the solution are controlled by a buffer solution added to the alcohol. The experimental conditions are given in Table 13.

Fraction II + III, which contains β - and γ -globulins, was subfractionated by the method of Nietschman *et al.*¹⁵⁰ in order to obtain a practically pure gamma globulin. The fraction II + III, which was separated from the supernatant fluid by centrifugation, was suspended in water without previous drying and extracted with a phosphate acetate buffer. From the solution β -globulin was first precipitated by the addition of 17 per cent alcohol at pH 5.1. A part of the γ -globulin was precipitated at the same time. The rest of the γ -globulin was then precipitated at pH 7.0 and an alcohol concentration of 25 per cent gave a product that was 90–95 per cent pure. As shown by many workers,^{8, 45, 113, 155} γ -globulin is not a homogeneous component. An attempt was made to separate the γ_1 - and γ_2 -globulin. This was done by precipitating with 25 per cent alcohol at -5°C and pH 5.4, when a fraction enriched with γ_2 was precipitated. After centrifugation the pH was adjusted to 7.2 and a γ_2 -rich frac-

TABLE 13 Fractionation scheme for human plasma (Cohn's method 6)

Fraction	Temp. $^{\circ}\text{C}$	Ionic strength	pH	Alcohol conc. %	Fractiona- tion yield %	Principal fraction
I	-3	0.14	7.2	8	5	fibrinogen
II + III	-5	0.09	6.8	25	27	$\beta + \gamma$ -globulin
IV: 1	-5	0.09	5.2	18	6	α -globulin
IV: 4	-5	0.09	5.8	40	7	$\alpha + \beta$ -globulin
V	-5	0.11	4.8	40	55	albumin

tion was obtained. According to Deutsch *et al.*⁴⁷ the iso-electric points of γ_1 and γ_2 are 5.7 and 7.3, respectively.

The yield of all the fractions was determined by Kjeldahl analysis of the nitrogen content of the precipitate and supernatants. Thus a nitrogen balance could be set up to check that the sum of the protein nitrogen fractions was equal to the total protein nitrogen of the serum. After precipitation of the fractions they were centrifuged at -5°C , the precipitate was suspended in saline and the suspension freeze dried. The dried preparations were stored in desiccators in a cold room ($+4^\circ\text{C}$).

INFRARED SPECTROSCOPY

The infrared spectra were recorded by means of a Perkin-Elmer spectrometer, Model 13, with a rock-salt prism, running single-beam.⁷⁸ The potassium pellet technique for sample preparation was used.

STATISTICAL METHODS

Method 1. Comparison of means (quantitative methods)

(a) t-test

Two means $\bar{x}$ and $\bar{y}$, based upon n_1 and n_2 observations on patients belonging to two groups A and B , were compared by means of the ordinary t -test

$$t = \frac{\bar{x} - \bar{y}}{s/\sqrt{\omega}},$$

where

$$s^2 = \frac{\sum (x - \bar{x})^2 + \sum (y - \bar{y})^2}{(n_1 - 1) + (n_2 - 1)}$$

and

$$\omega = \frac{n_1 n_2}{n_1 + n_2}.$$

(b) Combined t-test

In many cases, the groups A and B have different age compositions, so that when the factor studied is influenced by age, the means are not strictly comparable, and an uncritical application of the simple t -test may lead to false conclusions.

To avoid this situation, a combined t -test was used. The n_1 and n_2 observations were divided into k subgroups according to age, and means $\bar{x}_1, \dots,$

$\bar{x}_k$ and $\bar{y}_1, \dots, \bar{y}_k$ were calculated for each. Denote by $n_{11}, \dots, n_{1k}$ and $n_{21}, \dots, n_{2k}$ the number of observations in these groups. The hypothesis of no difference between A and B was tested by calculating a weighted mean of the differences between the means in the corresponding subgroups, using the expression:

$$t = \frac{\sum_{i=1}^k \omega_i (\bar{x}_i - \bar{y}_i)}{s \sqrt{\sum \omega_i}},$$

where

$$s^2 = \frac{\sum \sum (x - \bar{x}_i)^2 + \sum \sum (y - \bar{y}_i)^2}{\sum (n_{1i} - 1) + \sum (n_{2i} - 1)}$$

and

$$\omega_i = \frac{n_{1i} n_{2i}}{n_{1i} + n_{2i}}.$$

(c) Analysis of variance

The analyses of variance were performed according to usual methods (see, for example, Snedecor, 1956).

Method 2. Comparison of frequencies (qualitative methods)

(a) χ -test

The significance of the difference between two relative frequencies a_1/n_1 and a_2/n_2 was tested by calculating

$$\chi = \frac{a_1/n_1 - a_2/n_2}{\sqrt{pq/\omega}},$$

where

$$p = \frac{a_1 + a_2}{n_1 + n_2}, \quad q = 1 - p,$$

and ω has the same meaning as in 1(a) above. Continuity correction was used when the number of observations was small. If no real difference exists between the compared groups, χ is approximately normally distributed with mean 0 and standard deviation 1.

This test is mathematically identical with the usual χ^2 -test.

(b) Combined arc sin-test.

When the frequencies mentioned in (a) were influenced by the age compositions in the groups, a combined arc sin-test was used in order as far as possible to eliminate the age factor. As in 1(b), the total number of cases in the two groups was divided into k subgroups according to age, and the relative frequencies a_{1i}/n_{1i} and a_{2i}/n_{2i} were determined for each.

The significance was tested by calculating

$$t = \frac{\sum_{i=1}^k \omega_i [f(a_{1i}/n_{1i}) - f(a_{2i}/n_{2i})]}{\sqrt{\sum \omega_i}},$$

where

$$f(x) = 2 \sin^{-1} \sqrt{x},$$

and ω_i has the same meaning as in 1(b).

If no real difference exists between the groups, t is approximately normally distributed with mean 0 and standard deviation 1.

LEVELS OF SIGNIFICANCE

A difference was said to be *almost significant**, *significant*** or *highly significant****, when the corresponding value of P was less than 5, 1 or 0.1 per cent, respectively. When P was greater than 5 per cent, the difference was said to be not significant.

ERROR OF METHOD

A number of duplicate analyses of AST and ASta were extracted from the records of the routine analyses performed by the Central Bacteriological Laboratory of Stockholm, during the years 1949, 1951, and 1953. The values were analysed statistically by calculating the quotient of the largest and the smallest of each pair. The distribution of the quotients is reproduced in the following table.

		Number of duplicate analyses	Percentage of pairs with quotient less than or equal to				
			1.0	1.1	1.25	1.4	2.0
AST	327	58 ^a	67	71	86	99	100
ASta	297	55 ^a	66	73	80	99	100

^a In these cases, the duplicate values are equal.

CHAPTER IV

ANTISTREPTOLYSIN REACTION

The distribution of 1000 healthy pregnant women at term according to titre group was approximately normal, with the greatest number of cases falling in the 100–199 AST group (Fig. 1). The distribution was similar when the logarithms of the titre values were used*.

BIRTH WEIGHT

The relationship between the antistreptolysin titre and the mass of the foetus was tested by an analysis of variance for married and single primigravidae, and married and single multigravidae. The weight grouping was: 2501–3000 Gm, 3001–3500 Gm, 3501–4000 Gm and > 4000 Gm (Table 6). No correlation between the antistreptolysin titre and the mass of the foetus was established (Table 14).

MATERNAL AGE

The relationship between age and antistreptolysin titre in the marital status and parity groups was tested by an analysis of variance. The age grouping was: < 20 , 20–24, 25–29, 30–34, ≥ 35 years.

During the first months of pregnancy no significant differences between the age groups were found. On the other hand, during the latter half of gestation there was a clear difference between the age groups in respect of married primi- and multigravidae, significant values being obtained for all months except the 8th (Table 15).

The absence of a significant difference for the single subjects at any period of gestation may be ascribed in part to the smallness of the groups, since there was a significant difference for all groups combined in respect of the 10th month (Table 16).

MARITAL STATUS

In this examination of the relationship between the marital status and antistreptolysin titre the age factor was controlled (see *Method*). The values

* All statistical analysis were performed on the logarithmic values of the titres.

TABLE 14 AST versus birth weight

Months pregnant	Source of variation	Sum of squares	Degrees of freedom	Mean square	F
<i>Married primigravidae</i>					
VII	Between weight groups	2.066	14	0.148	1.1 ⁰
	Within " "	21.417	156	0.137	
VIII	Between weight groups	0.883	14	0.063	0.6 ⁰
	Within " "	19.037	188	0.101	
IX	Between weight groups	2.087	15	0.139	1.3 ⁰
	Within " "	24.172	225	0.107	
X	Between weight groups	1.899	14	0.136	1.4 ⁰
	Within " "	22.737	239	0.095	
<i>Single primigravidae</i>					
VII	Between weight groups	2.646	9	0.299	1.8 ⁰
	Within " "	7.335	45	0.163	
VIII	Between weight groups	0.590	8	0.074	0.1 ⁰
	Within " "	3.680	40	0.092	
IX	Between weight groups	1.110	11	0.101	0.9 ⁰
	Within " "	6.642	58	0.115	
X	Between weight groups	0.310	11	0.082	0.3 ⁰
	Within " "	7.375	73	0.101	
<i>Married multigravidae</i>					
VII	Between weight groups	2.912	13	0.224	1.5 ⁰
	Within " "	34.569	236	0.147	
VIII	Between weight groups	3.213	14	0.230	1.8 ⁰
	Within " "	30.051	239	0.126	
IX	Between weight groups	1.323	14	0.095	0.7 ⁰
	Within " "	48.582	336	0.145	
X	Between weight groups	1.191	14	0.079	0.6 ⁰
	Within " "	49.759	364	0.137	
<i>Single multigravidae</i>					
VII-VIII	Between weight groups	1.453	9	0.161	2.2 ⁰
	Within " "	1.020	14	0.073	
IX	Between weight groups	0.613	9	0.068	0.1 ⁰
	Within " "	8.864	17	0.521	
X	Between weight groups	2.928	9	0.325	1.1 ⁰
	Within " "	5.565	19	0.293	

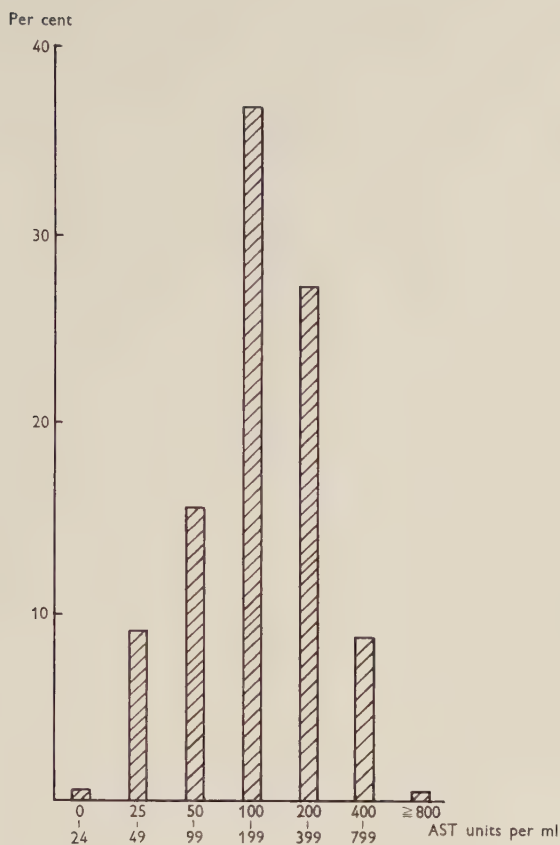


FIG. 1. Frequency distribution of AST for 1000 gravidae at term.



FIG. 2. AST for married primigravidae of age groups ≤ 24 and ≥ 30 years.

TABLE 15 AST versus age

Months pregnant	Source of variation	Sum of squares	Degrees of freedom	Mean square	F
<i>Married primigravidae</i>					
III	Between age groups	0.2925	4	0.073	0.7 ⁰
	Within " "	9.8135	97	1.012	
IV	Between age groups	1.1327	4	0.283	1.9 ⁰
	Within " "	13.2069	87	0.152	
V	Between age groups	1.3425	4	0.336	2.4 ⁰
	Within " "	14.3122	102	0.140	
VI	Between age groups	3.2298	4	0.808	7.7***
	Within " "	19.7354	188	0.105	
VII	Between age groups	1.5426	4	0.386	2.8**
	Within " "	23.4835	170	0.138	
VIII	Between age groups	0.7451	4	0.186	1.9 ⁰
	Within " "	19.9199	202	0.099	
IX	Between age groups	1.5486	4	0.387	3.6***
	Within " "	26.2590	240	0.109	
X	Between age groups	1.0916	4	0.273	2.8**
	Within " "	24.6356	253	0.097	
<i>Single primigravidae</i>					
III-IV	Between age groups	0.493	3	0.164	1.5 ⁰
	Within " "	3.917	35	0.112	
V-VI	Between age groups	0.215	4	0.054	0.5 ⁰
	Within " "	6.835	60	0.114	
VII	Between age groups	0.234	4	0.059	0.3 ⁰
	Within " "	9.981	54	0.185	
VIII	Between age groups	0.003	4	0.001	0.01 ⁰
	Within " "	4.230	48	0.089	
IX	Between age groups	0.616	4	0.154	1.4 ⁰
	Within " "	7.571	69	0.110	
X	Between age groups	0.278	4	0.070	0.8 ⁰
	Within " "	7.685	84	0.092	
<i>Married multigravidae</i>					
III	Between age groups	0.255	4	0.063	0.4 ⁰
	Within " "	21.245	145	0.147	

Table 15 (continued)

Months pregnant	Source of variation	Sum of squares	Degrees of freedom	Mean square	F
V	Between age groups	0.160	4	0.040	0.2 ⁰
	Within " "	28.888	152	0.190	
VI	Between age groups	0.963	4	0.241	2.0 ⁰
	Within " "	26.831	224	0.120	
VII	Between age groups	1.784	4	0.446	3.0**
	Within " "	37.572	249	0.151	
VIII	Between age groups	0.331	4	0.083	0.6 ⁰
	Within " "	33.264	253	0.132	
IX	Between age groups	1.591	4	0.398	2.8**
	Within " "	49.905	350	0.143	
X	Between age groups	1.616	4	0.404	3.0**
	Within " "	50.950	378	0.135	
<i>Single multigravidae</i>					
III-VI	Between age groups	0.437	4	0.109	1.3 ⁰
	Within " "	2.963	36	0.082	
VII-VIII	Between age groups	0.354	3	0.118	1.1 ⁰
	Within " "	2.473	23	0.108	
IX	Between age groups	1.851	4	0.463	1.3 ⁰
	Within " "	9.477	26	0.365	
X	Between age groups	0.609	4	0.152	0.5 ⁰
	Within " "	8.493	28	0.303	
IV	Between age groups	0.411	4	0.103	0.8 ⁰
	Within " "	16.669	135	0.123	

were consistently higher for single than married subjects. For primigravidae significant differences were found for the 8th to 10th lunar months. For multigravidae no significance was established for any stage of pregnancy (Table 17).

PARITY

This comparison between primi- and multigravidae was made for single and married subjects; age was controlled. In both cases the titre values were slightly higher for primigravidae, but for no period did they reach the level of significance (Table 18).

TABLE 16 AST according to age

Months pregnant	Maternal age	No. of cases	Mean AST	Percentage difference	<i>t</i>
<i>Married primigravidae</i>					
III	≤ 24	47	173	34	1.72 ⁰
	≥ 30	24	129		
IV	≤ 24	44	165	62	2.33*
	≥ 30	20	102		
V	≤ 24	55	165	74	2.68**
	≥ 30	15	95		
VI	≤ 24	90	178	78	4.18***
	≥ 30	45	100		
VII	≤ 24	90	136	20	1.00 ⁰
	≥ 30	32	113		
VIII	≤ 24	86	139	29	1.89 ⁰
	≥ 30	50	108		
IX	≤ 24	117	140	54	3.16**
	≥ 30	50	91		
X	≤ 24	114	141	41	2.72**
	≥ 30	57	100		
<i>Married multigravidae</i>					
III	≤ 24	33	150	15	0.68 ⁰
	≥ 30	75	131		
IV	≤ 24	31	173	28	1.68 ⁰
	≥ 30	75	135		
V	≤ 24	33	114	17	0.70 ⁰
	≥ 30	71	133		
VI	≤ 24	50	164	37	2.53*
	≥ 30	108	120		
VII	≤ 24	46	161	45	2.71**
	≥ 30	136	111		
VIII	≤ 24	51	146	26	1.60 ⁰
	≥ 30	136	116		
IX	≤ 24	72	157	44	3.29***
	≥ 30	173	109		
X	≤ 24	86	150	43	4.21***
	≥ 30	184	105		
<i>Single primi- and multigravidae</i>					
X	≤ 24	81	171	39	2.60**
	≥ 25	41	123		

TABLE 17 AST according to marital status

Months pregnant		Mean AST	Percentage difference	<i>t</i>
<i>Married and single primigravidae</i>				
III-IV	Married	149	17	0.30 ^o
	Single	175		
V-VI	Married	132	36	1.42 ^o
	Single	180		
VII	Married	117	26	0.84 ^o
	Single	148		
VIII	Married	125	37	2.31**
	Single	171		
IX	Married	120	53	3.20***
	Single	184		
X	Married	123	31	5.95***
	Single	161		
<i>Married and single multigravidae</i>				
III-IV	Married	132	25	1.21 ^o
	Single	165		
VII-VIII	Married	121	28	1.21 ^o
	Single	155		
IX	Married	117	6	0.09 ^o
	Single	124		
X	Married	119	13	0.39 ^o
	Single	135		

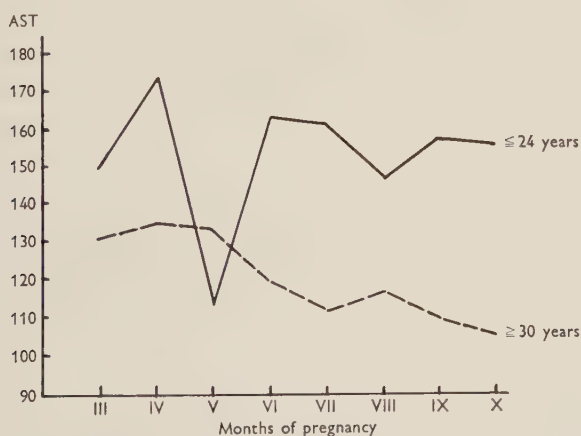
FIG. 3. AST for married multigravidae of age groups ≤ 24 and ≥ 30 years.

TABLE 18 AST according to parity

Months pregnant		Mean AST	Percentage difference	<i>t</i>
<i>Married primi- and multigravidae</i>				
III	Primigr.	155	9	0.52°
	Multigr.	142		
IV	Primigr.	142	2	1.00°
	Multigr.	139		
V	Primigr.	130	7	0.08°
	Multigr.	122		
VI	Primigr.	134	4	0.18°
	Multigr.	129		
VII	Primigr.	117	1	0.80°
	Multigr.	116		
VIII	Primigr.	125	0	0.95°
	Multigr.	125		
IX	Primigr.	120	3	1.36°
	Multigr.	117		
X	Primigr.	123	4	0.71°
	Multigr.	118		
<i>Single primi- and multigravidae</i>				
III-VI	Primigr.	178	8	0.41°
	Multigr.	165		
VII-VIII	Primigr.	159	3	0.13°
	Multigr.	155		
IX	Primigr.	184	48	1.29°
	Multigr.	124		
X	Primigr.	161	19	0.17°
	Multigr.	135		

STAGE OF PREGNANCY

The mean titre value tended to fall during the course of pregnancy (Figs. 2 and 3). Comparison of individual values for 300 cases of normal pregnancy at the beginning and end of gestation revealed a significantly higher value at the beginning***.

DISCUSSION

The specificity of the antistreptolysin reaction, for instance its property as a specific antibody reaction with β -haemolytic streptococci, has been dem-

onstrated in several studies^{131, 158, 204} However, some workers⁸⁸ have advanced the opinion that the reaction is not specific. Infections by β -haemolytic streptococci are common and there can be chronic infections of this type in the tonsils and lymph nodes without signs of disease. Moreover, such chronic infections can involve an elevation of the antistreptolysin titre. A study of a group of school children showed that haemolytic streptococci in the throat only influenced the titre where there was an inflammatory reaction, but that chronic infections of the respiratory tract were often followed by raised titres, especially if there was inflammation of the lymph nodes.²⁰⁴

Age. There seems to be general agreement that the age factor is of significance in respect of the AST and that the titres are higher in children than in adults.²⁰⁴

Marital status. The consistently higher values of the antistreptolysin titre for single than married subjects in this study may be due to the inferior social conditions of the single woman.

Parity. The slightly higher titre for primigravidae may be linked with the fact that many of the primigravidae are gainfully employed during pregnancy and thus come into more frequent contact with other people, the risk of infection thus being greater.

Stage of pregnancy. The significant decrease in the mean AST throughout pregnancy found in this study may have its explanation in several interrelated factors. During the last trimester the women are usually at home and are thus not so much exposed to the risk of respiratory infection as in the first and second trimesters.

During the latter part of pregnancy there may also be a hormonal depression of the antibody production. A recent study²⁵ has shown that the total 17-ketosteroids increase slightly throughout pregnancy. On the other hand, the plasma level of the 17-hydroxycorticosteroids did not show a steady increase during the course of pregnancy but displayed two maxima, one in the 5th and the other in the 8th month. Whether there is a relationship between the hormones of the placenta and adrenal cortex and the AST level it is difficult to decide, but it seems probable.

A third possible causal factor in the decrease in the mean AST is the accelerated transfer of these antibodies to the foetus during the latter half of pregnancy. The reduction in the antistreptolysin titre value during the course of pregnancy might also be ascribed to a number of conditions appearing during pregnancy, for instance hydraemia, which occurs during the later months.

CHAPTER V

ANTISTAPHYLOLYSIN REACTION

The distribution of 1000 healthy pregnant women at term according to titre group was approximately normal (Fig. 4). The titre values were below 0.7 ASt units per ml in 50 per cent of the cases, and below 1.4 units in 87.5 per cent.

BIRTH WEIGHT

A comparison similar to that for antistreptolysin titre was made for the birth weight, with an analysis of variance for the 7th to 10th months. Here, too, no significant differences were established for the single and married primigravidae, and single and married multigravidae. The birth weight grouping was as for AST (Table 19).

MATERNAL AGE

As in the case of antistreptolysin titre a variance analysis with respect to age was performed for the marital status and parity groups, the age grouping being as for AST analysis (Table 20). Significant differences were obtained for the 10th month in respect of married and the 8th month in respect of single primigravidae. The factor had a slightly greater influence in the married multigravidae group, significant differences being obtained for the 3rd, 7th and 9th months. The multigravidae group being small, no significant values were established. The significant values for the months in question are ascribable to generally higher titres for younger than older subjects (Figs. 5 and 6).

A study of the mean antistaphylolysin titres for the ≤ 24 and ≥ 30 year groups of married primigravidae and ≤ 30 and ≥ 30 year groups of married multigravidae disclosed a significant difference between the age groups only for the 10th month in respect of married primigravidae and for the 7th and 9th months in respect of married multigravidae. No significant difference was found for the single subjects, this being ascribable to some extent to the smallness of the groups (Table 21).

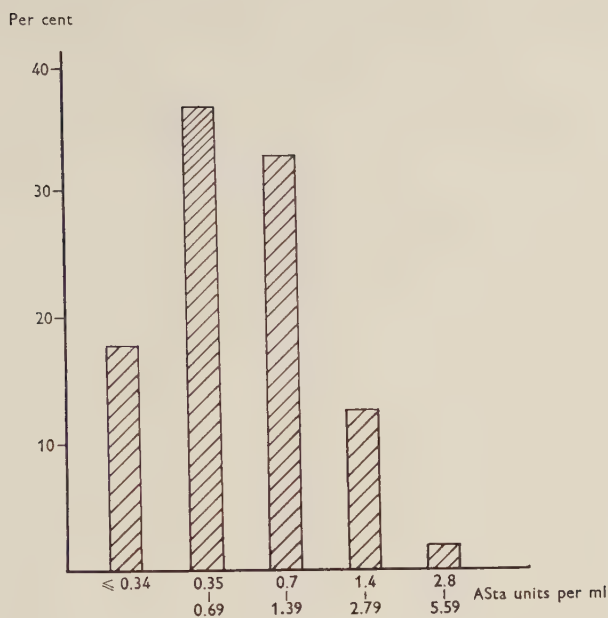


FIG. 4. Frequency distribution of ASTa for 1000 gravidae at term.

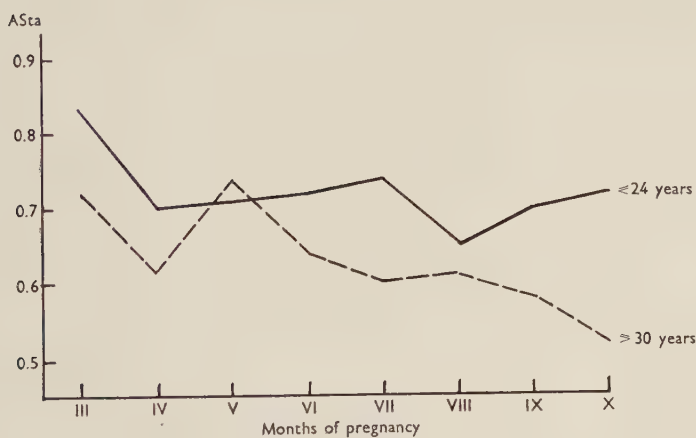


FIG. 5. ASTa for married primigravidae of age groups ≤ 24 and ≥ 30 years.

MARITAL STATUS

As in the case of AST, age was controlled in the analysis relating to marital status. No differences were found for any month in respect of either primi- or multigravidae (Table 22).

TABLE 19 *ASta versus birth weight*

Months pregnant	Source of variation	Sum of squares	Degrees of freedom	Mean square	F
<i>Married primigravidae</i>					
VII	Between weight groups	0.797	14	0.057	0.88°
	Within " "	10.185	157	0.065	
VIII	Between weight groups	0.648	14	0.046	0.66°
	Within " "	13.204	187	0.071	
IX	Between weight groups	0.798	15	0.053	0.78°
	Within " "	15.628	228	0.069	
X	Between weight groups	0.571	14	0.041	0.56°
	Within " "	17.165	241		
<i>Single primigravidae</i>					
VII	Between weight groups	0.972	9	0.108	2.25°
	Within " "	2.163	45	0.048	
VIII	Between weight groups	0.564	8	0.071	1.11°
	Within " "	2.668	42	0.064	
IX	Between weight groups	0.299	11	0.027	0.33°
	Within " "	4.826	59	0.082	
X	Between weight groups	0.780	11	0.071	0.88°
	Within " "	5.777	72		
<i>Married multigravidae</i>					
VII	Between weight groups	0.584	13	0.045	0.55°
	Within " "	19.031	235	0.081	
VIII	Between weight groups	0.829	14	0.095	0.13°
	Within " "	16.644	236	0.071	
IX	Between weight groups	0.377	14	0.027	0.45°
	Within " "	20.366	340	0.060	
X	Between weight groups	1.106	15	0.074	1.19°
	Within " "	22.447	362	0.062	
<i>Single multigravidae</i>					
VII	Between weight groups	0.586	10	0.059	1.1°
	Within " "				
VIII	Between weight groups	0.865	16	0.054	
	Within " "				
IX	Between weight groups	0.700	8	0.088	1.73°
	Within " "	0.750	15	0.050	
X	Between weight groups	0.780	11	0.071	0.88°
	Within " "	5.777	72	0.080	

TABLE 20 *AS_ta versus age*

Months pregnant	Source of variation	Sum of squares	Degrees of freedom	Mean square	<i>F</i>
<i>Married primigravidae</i>					
III	Between age groups	0.287	4	0.072	0.74 ^o
	Within " "	9.446	97	0.097	
IV	Between age groups	0.353	4	0.088	1.16 ^o
	Within " "	6.654	87	0.076	
V	Between age groups	0.043	4	0.010	0.09 ^o
	Within " "	11.076	101	0.109	
VI	Between age groups	0.370	4	0.093	1.43 ^o
	Within " "	11.967	185	0.065	
VII	Between age groups	0.577	4	0.144	2.25 ^o
	Within " "	10.982	171	0.064	
VIII	Between age groups	0.081	4	0.020	0.29 ^o
	Within " "	13.852	201	0.069	
IX	Between age groups	0.430	4	0.107	1.59 ^o
	Within " "	16.426	243	0.068	
X	Between age groups	0.919	4	0.230	3.23**
	Within " "	18.186	259	0.071	
<i>Single primigravidae</i>					
III-IV	Between age groups	0.331	3	0.110	1.58 ^o
	Within " "	2.435	35	0.070	
V-VI	Between age groups	0.403	4	0.101	1.16 ^o
	Within " "	5.089	59	0.086	
VII	Between age groups	0.359	4	0.090	1.55 ^o
	Within " "	3.135	54	0.048	
VIII	Between age groups	0.934	4	0.233	3.61**
	Within " "	3.232	50	0.065	
IX	Between age groups	0.272	4	0.068	0.93 ^o
	Within " "	5.125	70	0.073	
X	Between age groups	0.108	4	0.027	0.34 ^o
	Within " "	6.557	83	0.079	
<i>Married multigravidae</i>					
III	Between age groups	0.853	4	0.211	3.91***
	Within " "	7.728	143	0.054	
IV	Between age groups	0.385	4	0.096	1.35 ^o
	Within " "	9.862	138	0.071	

TABLE 20 (continued)

Months pregnant	Source of variation	Sum of squares	Degrees of freedom	Mean square	F
V	Between age groups	0.094	4	0.024	0.33 ^o
	Within " "	11.218	157	0.072	
VI	Between age groups	0.221	4	0.055	0.80 ^o
	Within " "	15.475	223	0.069	
VII	Between age groups	1.044	4	0.261	3.30**
	Within " "	19.615	248	0.079	
VIII	Between age groups	0.455	4	0.114	1.63 ^o
	Within " "	17.473	250	0.070	
IX	Between age groups	0.715	4	0.179	3.03**
	Within " "	20.743	354	0.059	
X	Between age groups	0.250	4	0.062	1.00 ^o
	Within " "	23.553	377	0.062	
<i>Single multigravidae</i>					
III-VI	Between age groups	0.499	4	0.127	1.92 ^o
	Within " "	1.377	21	0.066	
VII-VIII	Between age groups	0.266	3	0.089	1.60 ^o
	Within " "	1.451	26	0.056	
IX	Between age groups	0.161	4	0.040	0.63 ^o
	Within " "	1.449	23	0.063	
X	Between age groups	0.435	4	0.109	1.73 ^o
	Within " "	1.693	27	0.063	

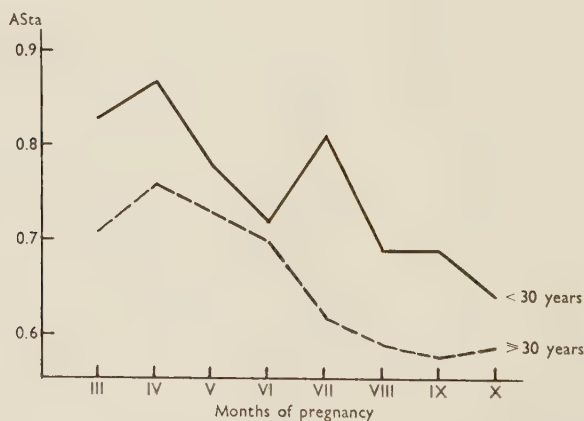


FIG. 6. ASa for married multigravidae of age groups < 30 and ≥ 30 years.

TABLE 21 ASta according to age and parity

Months pregnant	Maternal age	No. of cases	Mean ASta	Percentage difference	<i>t</i>
<i>Married primigravidae</i>					
III	≤ 24	47	0.83	15	0.79 ⁰
	> 30	24	0.72		
IV	≤ 24	44	0.70	13	0.70 ⁰
	> 30	20	0.62		
V	≤ 24	55	0.71	4	0.22 ⁰
	> 30	14	0.74		
VI	≤ 24	88	0.72	13	1.13 ⁰
	> 30	46	0.64		
VII	≤ 24	91	0.74	23	1.57 ⁰
	> 30	32	0.60		
VIII	≤ 24	86	0.65	7	0.61 ⁰
	> 30	48	0.61		
IX	≤ 24	118	0.70	21	1.77 ⁰
	> 30	51	0.58		
X	≤ 24	115	0.72	38	3.73***
	> 30	58	0.52		
<i>Married multigravidae</i>					
III	< 30	75	0.83	17	1.62 ⁰
	≥ 30	73	0.71		
IV	< 30	67	0.87	14	1.21 ⁰
	≥ 30	76	0.76		
V	< 30	92	0.78	7	0.41 ⁰
	≥ 30	70	0.73		
VI	< 30	120	0.72	3	0.49 ⁰
	≥ 30	108	0.70		
VII	< 30	118	0.81	31	3.33***
	≥ 30	135	0.62		
VIII	< 30	122	0.69	17	2.11*
	≥ 30	133	0.59		
IX	< 30	183	0.69	19	3.06**
	≥ 30	176	0.58		
X	< 30	201	0.64	8	1.41 ⁰
	≥ 30	181	0.59		

TABLE 21 (continued)

Months pregnant	Maternal age	No. of cases	Mean ASta	Percentage difference	<i>t</i>
<i>Married and single primi- and multigravidae</i>					
III	< 30	153	0.83	17	2.02*
	≥ 30	97	0.71		
IV	< 30	139	0.80	10	1.09 ^o
	≥ 30	96	0.73		
V	< 30	184	0.75	1	0.05 ^o
	≥ 30	84	0.74		
VI	< 30	264	0.73	7	1.12 ^o
	≥ 30	154	0.68		
VII	< 30	262	0.73	18	2.66**
	≥ 30	167	0.62		
VIII	< 30	171	0.68	19	2.57*(*)
	≥ 30	139	0.57		
IX	< 30	248	0.70	23	3.66***
	≥ 30	186	0.57		
X	< 30	403	0.65	14	2.88**
	≥ 30	239	0.57		

PARITY

A comparison was made between single and married subjects with age controlled. In neither group were significant values found, from which it may be concluded that no account need be taken of the number of pregnancies when determining the antistreptolysin titre.

TABLE 22 ASta according to marital status, age controlled; 10th month

		No. of cases	Mean ASta	<i>t</i>
Primigravidae	Married	260	0.64	0.11 ^o
	Single	88	0.68	
Multigravidae	Married	382	0.61	0.83 ^o
	Single	32	0.56	

The antistaphylolysin titre showed a definite tendency to fall during the course of pregnancy. Comparison of the individual values for 200 cases of normal pregnancy at the beginning and end of pregnancy revealed a significantly higher value at the beginning (Figs. 5 and 6).

DISCUSSION

The antistaphylolysin titre in normal subjects and various pathologic conditions has been studied by several workers.^{122, 124, 192, 205} The relationship between *Staphylococcus aureus* in the nose and throat and the AS_ta was also closely investigated.¹⁵⁹

Age. Lower titres were found in children than in adults. In a study²⁰⁵ of 109 school children of ages varying from 7 to 16 years, 23 per cent had titre values of less than 0.56 units per ml, 72 per cent values between 0.56 and 2.0 units and 5 per cent had higher values. The corresponding values in the present study were 50.9, 47.5 and 1.6 per cent (Table 53). The AS_ta values in the 10th month of pregnancy were thus significantly lower than in the quoted group of healthy school children. In the present study the mean titre value was generally higher in the lower age groups, though the difference was significant only for the last trimester.

Stage of pregnancy. The reduction in the antistaphylolysin titre value during the course of pregnancy might be ascribed to a number of conditions appearing during pregnancy, for instance hydraemia, which occurs during the later months. Transference of antibodies from the mother to the foetus may also be of significance. Moreover, there is often an attempt to avoid exposure to infections, the mother being so advised at the maternity centre. As in the case of the AS_t, it is possible that the AS_ta level is influenced by placental hormones and corticosteroids.

CHAPTER VI

BACT. COLI AGGLUTINATION TEST

The distribution of 215 healthy pregnant women at the 9th to 10th month according to *Bact. coli* agglutination titre groups (antigens O and H) was rather skew. The logarithms of the titres gave a symmetric distribution for the O and a skew distribution for H. The series was too small to provide a clear impression of the frequency distribution.

BIRTH WEIGHT

No study was made of the birth weight as the series was too small, and as no influence of this factor was found in the AST and ASta studies.

MATERNAL AGE

In respect of O antigen no agglutination was obtained with a 1:20 dilution for about 20 per cent of the subjects during the various stages of pregnancy, this applying to the ≤ 24 and ≥ 25 year groups. In respect of the antigen H no agglutination was obtained towards the end of gestation for the same dilution in over 50 per cent of the subjects of the lower age group. The corresponding figure for the higher age group was 23.5 per cent. The difference was highly significant. (Table 23.) A comparison of the number of cases in the two age groups for whom agglutination against O and/or H antigen was obtained at a serum dilution $\geq 1:320$ revealed no significant difference (Table 24).

PARITY

No significant differences were established in a corresponding comparison between married primi- and multigravidae.

STAGE OF PREGNANCY

The *Bact. coli* agglutination titre against O and H antigen showed a definite tendency to fall during the course of pregnancy. Comparison of the individual values for 83 cases at the beginning and end of pregnancy showed a significantly higher value at the beginning**.

TABLE 23 *Bact. coli* agglutination titres according to age for married and single primigravidae at various periods of gestation

Age group	Months pregnant	No aggl.		Agglutination				
		No.	%	≥ 1 : 40 No.	≥ 1 : 80 No.	≥ 1 : 160 No.	≥ 1 : 320 No.	≥ 1 : 640 No.
<i>Married primigravidae</i>								
<i>Antigen O</i>								
≤ 24	III-V	15	19	63	63	51	20	4
	VI-VIII	14	21	54	54	36	15	2
	IX-X	20	24	64	64	46	18	8
≥ 25	III-V	14	22	49	49	40	18	2
	VI-VIII	23	27	62	62	47	15	3
	IX-X	19	22	68	68	43	23	6
<i>Antigen H</i>								
≤ 24	III-V	21	32	44	44	16	6	3
	VI-VIII	30	44	38	38	16	6	—
	IX-X	45	52	42	42	21	4	2
25	III-V	17	25	50	50	26	4	1
	VI-VIII	36	44	45	45	29	6	—
	IX-X	27	24	88	88	25	6	1
<i>Single primigravidae</i>								
<i>Antigen O</i>								
≤ 24	III-V	6	26	17	17	13	6	—
	VI-VIII	3	12	22	22	15	5	2
	IX-X	5	18	23	23	20	9	3
25	III-V	1	10	9	9	7	2	—
	VI-VIII	2	17	10	10	9	1	—
	IX-X	3	25	9	9	4	2	2
<i>Antigen H</i>								
≤ 24	III-V	10	40	15	15	9	3	—
	VI-VIII	7	28	18	18	12	4	2
	IX-X	9	31	20	20	14	5	1
≥ 25	III-V	3	30	7	7	3	1	—
	VI-VIII	1	10	9	9	7	1	1
	IX-X	5	42	7	7	4	2	—

TABLE 24 *Bact. coli* agglutination titres $\geq 1:320$ according to age; married and single primigravidae

	Age group ≤ 24 years		Age group ≥ 25 years		Difference
	No. cases	%	No. cases	%	(%)
<i>Married primigravidae</i>					
<i>Antigen O</i>					
III-V	20	26	18	29	3 ^o
VI-VIII	15	22	15	18	4 ^o
IX-X	18	21	23	26	5 ^o
<i>Antigen H</i>					
III-V	6	9	4	6	3 ^o
VI-VIII	6	8	6	7	1 ^o
IX-X	4	5	6	5	0
<i>Single primigravidae</i>					
<i>Antigen O</i>					
III-V	6	26	2	20	6 ^o
VI-VIII	5	20	1	8	12 ^o
IX-X	9	32	2	16	16 ^o
<i>Antigen H</i>					
III-V	3	12	1	10	2 ^o
VI-VIII	4	16	1	10	6 ^o
IX-X	5	17	2	17	0

CHAPTER VII

DYE AND COMPLEMENT FIXATION TESTS

The level of circulating antibodies against *Toxoplasma* was examined by the dye (DT) and complement fixation (CF) tests in 547 gravaidee presenting no complications and giving birth to healthy children (Tables 25 and 26).

TABLE 25 Variation in response to dye test of 547 gravaidee, according to age

Maternal age	Dye test negative		Dye test positive						Total No.
			1:10		1:50		≥ 1:250		
	No.	%	No.	%	No.	%	No.	%	
≤ 24	145	70.1	28	13.5	23	11.1	11	5.3	207
25-29	90	58.8	29	19.0	29	19.0	5	3.2	153
30-34	58	50.9	30	26.3	22	19.3	4	3.5	114
≥ 35	35	48.0	23	31.5	15	20.5			73
Total	328	60.0	110	20.1	89	16.3	20	3.6	547

TABLE 26 Variation in response to complement fixation test of 547 gravaidee, according to age

Maternal age	CF test negative		Complement fixation test positive								Total No.
			1:7.5		1:15		1:30		≥ 1:60		
	No.	%	No.	%	No.	%	No.	%	No.	%	
≤ 24	149	72.7	24	11.7	18	8.8	10	4.9	4	1.9	205
25-29	105	67.7	17	11.0	19	12.3	11	7.1	3	1.9	155
30-34	69	60.5	13	11.5	16	14.0	12	10.5	4	3.5	114
≥ 35	48	65.8	12	16.4	6	8.2	7	9.6			73
Total	371	67.8	66	12.1	59	10.8	40	7.3	11	2.0	547

BIRTH WEIGHT

No study was made of birth weight as no influence of this factor was found in the antistreptolysin and antistaphylolysin tests.



FIG. 7. Frequency distribution of DT positive for various age groups.

MATERNAL AGE

Dye test. The number of positive reactions increased with age, a comparison of the ≤ 24 and ≥ 30 year groups showing a significant difference for the dilution 1:10 but not for $\geq 1:50$ (Table 27).

TABLE 27 Response to dye test and complement fixation test according to age group

		≤ 24 years		≥ 30 years		Difference
		No.	%	No.	%	(%)
<i>Dye test</i>						
Negative		145	70.1	93	49.7	20.4***
Positive : titre	1 : 10	28	13.5	53	28.3	14.8***
	≥ 1 : 50	34	16.4	41	21.9	5.5 ⁰
<i>Complement fixation test</i>						
Negative		149	72.7	117	62.6	10.1*
Positive : titre	1 : 7.5	24	11.7	25	13.4	1.7 ⁰
	≥ 1 : 30	14	6.8	23	12.3	5.5*

Complement fixation test. There was a tendency towards an increase in the number of positive responses with age. In a comparison between the ≤ 24 and ≥ 30 year groups the difference at dilution 1:7.5 was not significant and at $\geq 1:30$ almost significant (Table 27).

MARITAL STATUS

The low number of single subjects for which the pregnancy was normal precluded a reliable study of the relationship between the titres and marital status. A tentative analysis revealed no significant differences between the number of positive responses to the dye test obtained for single and married gravaide.

PARITY

For neither the dye nor the complement fixation test was a significant difference found in respect of primi- and multigravidae, when the age factor was controlled (Tables 28 and 29).

TABLE 28 Positive response to dye test according to marital status and parity

Maternal age	Total no.	DT ≥ 1 : 50		Total no.	DT ≥ 1 : 50		t (arc sin test)
		No.	%		No.	%	
		<i>Single</i>			<i>Married</i>		
< 30	22	6	27.3	338	62	18.3	1.66°
≥ 30	5	3	60.0	182	38	20.9	
Total cases	27	9	33.3	520	100	19.2	
		<i>Primigravidae</i>			<i>Multigravidae</i>		
≤ 24	130	18	13.8	77	16	20.8	0.61°
25-29	58	11	19.0	95	23	24.2	
≥ 30	46	13	28.3	141	28	19.9	
Total cases	234	42	17.8	313	67	21.4	

TABLE 29 Positive response to complement fixation test according to parity; age controlled

Maternal age	Total no.	CF ≥ 1 : 15		Total no.	CF ≥ 1 : 15		t (arc sin test)
		No.	%		No.	%	
		<i>Primigravidae</i>			<i>Multigravidae</i>		
≤ 24	142	23	16.2	63	9	14.3	1.1°
25-29	61	10	16.4	94	23	24.5	
≥ 30	44	18	40.9	143	27	18.9	
Total cases	247	51	20.6	300	59	19.7	

STAGE OF PREGNANCY

Dye test

The percentage of positive titres was higher in the older age groups; moreover, in each group there was a slight tendency towards an increase with the period of gestation (Tables 30 and 31; Fig. 8). Comparison of the positive responses at the beginning and end of pregnancy showed no significant difference.

TABLE 30 Response of 547 gravidae to dye test at various periods of gestation

Months pregnant	Maternal age	Dye test negative		Dye test positive						Total
				1:10		1:50		≥ 1:250		
		No.	%	No.	%	No.	%	No.	%	
II	≤ 24	58	77.3	9	12.0	5	6.7	3	4.0	75
	25-29	37	68.5	11	20.4	4	7.4	2	3.7	54
IV	≥ 30	38	59.4	21	32.8	5	7.8	—		64
V	≤ 24	81	77.1	11	10.5	11	10.5	2	1.9	105
	25-29	53	67.9	13	16.7	11	14.1	1	1.3	78
VI	≥ 30	47	48.5	38	39.2	11	11.3	1	1.0	97
VII	≤ 24	76	75.2	10	9.9	9	8.9	6	6.0	101
	25-29	43	61.4	13	18.6	12	17.1	2	2.9	70
VIII	≥ 30	56	58.9	27	28.4	10	10.5	2	2.1	95
IX	≤ 24	122	74.4	20	12.2	16	9.8	6	3.6	164
	25-29	82	62.6	30	22.9	17	13.0	2	1.5	131
X	≥ 30	86	57.3	39	26.0	22	14.7	3	2.0	150

TABLE 31 Positive response to dye test and complement fixation test at beginning and end of pregnancy, according to age

Maternal age	II-IV months pregnant			IX-X months pregnant			<i>t</i>	
	Total no.	No.	%	Total no.	No.	%		
		<i>DT</i> ≥ 1:50			<i>DT</i> ≥ 1:50			
≤ 24	75	8	10.7	164	22	13.4	1.7 ^o	
25-29	54	6	11.1	131	19	14.5		
≥ 30	64	5	7.8	150	25	16.7		
Total cases	193	19	9.8	445	66	14.8		
		<i>CF</i> ≥ 1:30			<i>CF</i> ≥ 1:30			
≤ 24	67	3	4.5	128	10	7.8	0.26 ^o	
25-29	54	3	5.6	131	5	3.8		
≥ 30	66	5	7.6	151	7	4.6		
Total cases	187	11	5.9	410	22	5.4		

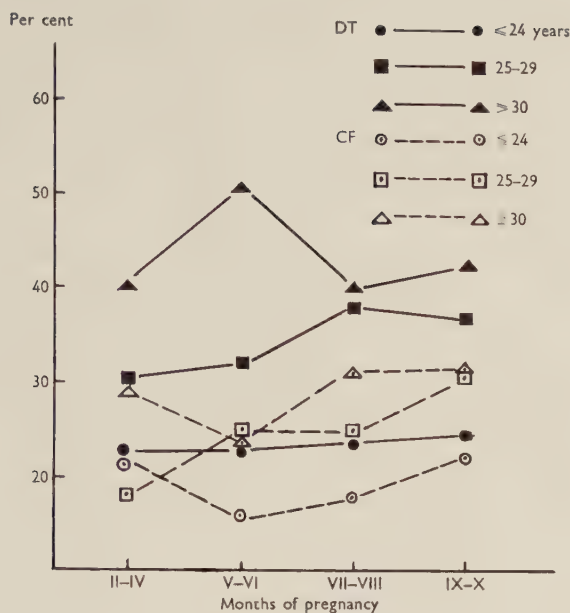


FIG. 8. Variation in the frequency of dye test and complement fixation test titres during the course of pregnancy.

Complement fixation test

The percentage of positive titres was higher in the older age groups, the number being slightly higher at term. Comparison of the number of positive responses for the dilution $\geq 1:30$ at the beginning and end of gestation, with age controlled, revealed no significant difference (Table 31 & 32).

A high correlation was found between the dye and complement fixation test titres (Table 33).

Change in response to dye test during gestation

In 6 cases a dye test and/or complement fixation test changed from negative to positive during the course of pregnancy. In these cases there may have been a subclinical infection. In none was any untoward effect on the child evident during the first weeks of life. The last case in the Table 34 is of particular interest. In the 6th lunar month obscure abdominal pains were experienced, and at the maternity centre some form of threatened abortion was suspected, although there was no external haemorrhage. Progesteron and rest were prescribed, and after about one month the patient felt better; the subsequent course of pregnancy was normal. Examination of the placenta revealed no infarction nor sign of former haemorrhage. There was no sign of lymphadenopathy. The subsequent course of pregnancy was uneventful.

TABLE 32 Response of 547 gravidae to complement fixation test at various periods of gestation

Months pregnant	Maternal age	CF negative		CF positive								Total
		No.	%	1:7.5		1:15		1:30		≥ 1:60		
				No.	%	No.	%	No.	%	No.	%	
II	≤ 24	52	77.6	5	7.5	7	10.4	2	3.0	1	1.5	67
	25-29	44	81.5	2	3.7	5	9.3	3	5.6	—	—	54
IV	≥ 30	47	71.2	6	9.1	8	12.1	5	7.6	—	—	66
V	≤ 24	90	84.9	2	1.9	6	5.7	5	4.7	3	2.8	106
	25-29	56	74.7	7	9.3	7	9.3	4	5.3	1	1.3	75
VI	≥ 30	70	75.3	6	6.5	13	14.0	2	2.1	2	2.1	93
VII	≤ 24	83	82.1	4	4.0	10	9.9	2	2.0	2	2.0	101
	25-29	54	75.0	6	8.3	7	9.7	4	5.6	1	1.4	72
VIII	≥ 30	66	70.2	12	12.8	12	12.8	2	2.1	2	2.1	94
IX	≤ 24	128	77.1	17	10.3	11	6.6	6	3.6	4	2.4	166
	25-29	93	71.0	21	16.0	12	9.2	3	2.3	2	1.5	131
X	≥ 30	106	70.2	19	12.6	19	12.6	5	3.3	2	1.3	151

TABLE 33 Relation between dye test and complement fixation test titres during normal pregnancy

Complement fixation test	Dye test negative		Dye test positive						Total no.	
			1 : 10		1 : 50		≥ 1 : 250			
			No.	%	No.	%	No.	%	No.	%
CF negative	1049	61.5	173	10.0	32	1.9	5	0.3	1259	73.8
CF positive										
1 : 7.5	54	3.2	74	4.3	44	2.6	2	0.1	174	10.2
1 : 15	24	1.4	66	3.9	69	4.0	6	0.3	165	9.6
1 : 30	5	0.3	18	1.1	36	2.1	9	0.5	68	4.0
1 : 60	—	—	—	—	6	0.3	35	2.1	41	2.4
Total	1132	66.3	331	19.4	187	11.0	57	3.3	1707	100.0

To judge from the findings for this series the chance of contracting toxoplasmosis during pregnancy is about 1 per cent—a figure that should be regarded as a minimum. In the normal group of 547 cases there were a further 9 subjects with a dye test titre of $\geq 1:250$ for whom there may have been infection during pregnancy; however, the tests were not performed early enough to indicate when the infection may have taken place. These 9 cases together with one of toxoplasmosis confirmed in connection with an abortion three

TABLE 34 Change in response to dye test from negative to positive during gestation

Record no.	Maternal age	Clinical data	Toxoplasmosis titres (DT and/or CF) at various periods of gestation							Cord blood
			III-IV	V	VI	VII	VIII	IX	X	
956	21	Primigravida. Normal preg. and delivery, boy 2910 Gm normal. Puerperium: trace of albumin during first week.	—	—	—	neg.	neg.	neg.	1:250	1:250
1552	34	1944 delivery normal. Present pregnancy and delivery normal, boy 3000 Gm. Puerperium normal.	neg.	—	—	—	—	neg.	1:50	1:50
1688	30	1944, 1947 deliveries normal: 1949 abortion in 3rd month. Present pregnancy and delivery normal, boy 3940 Gm.	—	—	—	—	neg.	1:50	1:500	1:250
2777	26	Primigravida. Pregnancy and delivery normal, girl 3830 Gm. Puerperium normal.	neg.	—	—	—	neg.	1:15	1:30	1:30
3139	27	1946 abortion in 6th month. 1946 densification in left lung. Present pregnancy and delivery normal, boy 3100 Gm. Puerperium. Lungs normal.	—	neg.	—	—	—	—	1:50	1:50
3880	30	1947 delivery normal. Pregnancy normal until 5-6th months: back and abdominal pains; no haemorrhage. Condition improved after one month's rest. Delivery normal, girl 2900 Gm. Placenta normal. Puerperium normal.	neg.	—	1:2000	1:2000	—	1:1000	1:1000	1:1000
			neg.	—	—	1:120	—	1:60	1:60	1:60

years previously have been entered in Table 35. By way of illustrating that a high antibody titre does not necessarily result in injury to the foetus a case is described in which toxoplasmosis was contracted and high titres recorded a short time before pregnancy.

CASE 2663 R. M. 17 years of age. Menarche at 14 years of age. Regular periods 4/28 days. 7.3.50 acute indisposition with temperature of 40°, continuing for some days. 10.3.50 admitted to South Stockholm Hospital. General condition normal. Complained of headache, no other symptoms. Small papulovesicular eruptions on the body. No signs of lymphadenopathy. Heart and lungs normal. Lung radiograph normal. Lumbal puncture revealed no pathologic signs. White cell count 3000.

Diagnosis: Virus infection?

12.3.50 Temperature: morning 39.5, afternoon 38.9. The eruption had spread to neck and face.

Blood tests: Paul-Bunnell negative. Cold agglutination test negative. Widal's reaction negative. Wassermann reaction negative in blood and spinal fluid. Sternal puncture: suspected mononucleosis.

15.3 tempature falling. Small cervical inflamed lymph nodes.

22.3 afebrile Eruption cleared up.

3.4 fever returned. Tachycardia. No local symptoms.

6.4 temperature 39°. Lymphadenitis, almond-sized nodes on right side of neck. Recovered over one month.

Serologic examination for toxoplasmosis

Date	DT	CF
5.4.1950	1:250	—
27.4	1:8000	1:64
15.5	1:4000	1:3840
7.12	Last normal period	
18.12	1:1000	1:240
12.3	1:250	1:15
9.4	1:50	1:480
21.6	1:500	1:240
25.8	1:250	1:120
15.9 Delivery		
Maternal blood	1:500	1:60
Cord blood	1:500	1:60

The child, a boy, weighed 3740 Gm and presented no signs of toxoplasmosis.

DISCUSSION

The specificity of the dye and complement fixation tests for toxoplasmosis has been commented on by several investigators.^{59, 73, 85, 200} However, some workers¹³ have suggested that infections with Sarcocytis, Trichomonas and Try-

Record Maternal no. age		Clinical data	Toxoplasmosis titres (DT and/or CF) at various periods of gestation									
			III-IV	V	VI	VII	VIII	IX	X	G	P	
1492	21	Primigravida. Mild toxæmia during last two weeks of pregnancy. Delivery normal, boy 3440 Gm. No signs of toxoplasmosis.	—	—	1:50 neg.	—	1:250 1:7.5	1:50 1:15	1:50 1:30	1:50 + —		
1662	18	One year earlier girl, 3450 Gm. Present pregnancy and delivery normal, boy 2950 Gm. WR neg. Rh neg. no immun.	—	—	1:10 1:30	—	1:50 1:30		1:250 1:30	1:250 1:30		
2227	19	One year earlier, normal delivery, girl 2900 Gm. Present pregnancy and delivery normal, boy 2950 Gm.	1:10 1:15	—	—	1:250 + 1:15	—	1:50 1:15	1:50 1:15	1:250 1:30		
1687	25	1947 delivery normal, girl 3500 Gm, 1948 abortion in 5th month, followed by acute toxoplasmosis with high titres. Present pregnancy and delivery, 1951, normal, boy 3980 Gm.			1:250 1:60				1:50 1:240	1:50 —		
2756	23	Primigravida. Present pregnancy and delivery normal, boy 3370 Gm.	1:250 1:480	—	1:2000 1:220	—	1:2000 1:480	1:8000 1:960	1:4000 1:120	1:4000 1:120		
1478	29	1950 delivery normal, boy 3680 Gm. Present pregnancy and delivery normal, girl 3150 Gm.	—	—	—	1:50 1:30	—	1:250 1:240	1:1000 1:240	1:1000 1:240	1:250 + 1:30 + *	
2437	20	1948 delivery normal, boy 4100 Gm. 1949 abortion in 5th month. 1950 abortion with high fever in 2nd month. Present pregnancy—anaemia and discharge. Delivery normal, 1951, girl 4870 Gm.	1:10 neg.	—	—	1:250 1:7.5 +	—	—	1:50 neg.	1:50 neg.		
2133	27	Primigravida. In 4th month abdominal pains. No fever observed at hospital for one week. Course and delivery then normal, 3200 Gm.	1:250 neg.	—	—	—	1:10 1:15	—	1:50 1:15	1:50 —		
2348	27	Primigravida. One year previously rheumatoid arthritis. Pregnancy and delivery normal, girl 2910 Gm. Three weeks later acute salpingitis and lupus erythematoses diss (?). Four years later normal delivery, girl 2830 Gm.	—	1:250 1:480	—	—	1:250 1:480	1:500 1:120	1:1000 1:240	1:500 + 1:240		
2305	20	Primigravida. Pregnancy and delivery normal, girl 3030 Gm.	—	—	1:250 neg.	—	—	—	1:50 + 1:30	1:1000 1:30		

* 3 weeks postpartum.

panosoma can give cross-reactions in the dye test, though this has not been confirmed in later studies²⁰⁰.

In a survey of toxoplasmosis⁷³, the experience of the dye test has been summarized thus: A positive reaction is proof of current or past infection with *Toxoplasma*. In the acute stage of the infection the titre rises to a value of 250 or more. After 3–6 months the values begin to fall; persistent high values indicate continued antigen stimulation (for instance, there may be focal infection). Anamnestic reactions sometimes occur, when a previously weak or moderate serologic reaction may become more pronounced in connection with an infection of another type. There will probably be no new formation of antibodies, but an outflow from depots in the cells. In these cases, rises in the titres are generally slight or of brief duration.

Can pregnancy give rise to non-specific increases in the titres of various types? It has been maintained that pregnancy gives rise to changes in the body that are reminiscent of reactions occurring in infection.

In a study of toxoplasmosis²²⁸ a positive dye test was found in 48.5 per cent of 270 pregnant women; the corresponding figure for a non-pregnant group of blood donors was 37.7 per cent, the difference being significant. Other workers³⁵ have also found that the incidence of positive reactions among women who have had a normal delivery was unusually high, and it was concluded that pregnancy can obviously result in increased titres. It is possible that this conclusion may be right, but as the investigators evidently did not perform repeated tests during pregnancy the increased higher titre frequency might equally well be a manifestation of a more marked susceptibility to *Toxoplasma* infection during pregnancy. In the material of the present study, which was followed with repeated tests during the various stages of pregnancy, there was no significant displacement of the dye test titres, if age was controlled. In contrast to the case of AST and AS_{ta}, where there was a decrease in the titres, in the dye test there was a tendency towards an increase in the titre values during the course of pregnancy. The question of whether pregnancy gives an anamnestic reaction can still not be answered with confidence, nor can the question of whether pregnancy gives rise to increased susceptibility to toxoplasmosis. In the present material at least one per cent of the cases contracted toxoplasmosis during pregnancy. There is no available information on the corresponding condition for non-pregnant women of the same age. The incidence during pregnancy seems to vary from one region to another. In a paper from the United States the author⁶⁰ states: "We have not been able to detect infection, as evidenced by a change in antibody status, in about 600 pregnant women followed to term."

It is still unclear what causes the infection of the foetus when the mother has toxoplasmosis during pregnancy. It is evidently only in a small number of cases of toxoplasmosis in pregnancy that the foetus is infected, and probably

as a result of parasitaemia. Some workers maintain that infection of the placenta occurs, from which the foetus will then contract the disease. It was however only in a few cases that such infection of the placenta could be demonstrated. As defects in the placenta are not uncommon,¹⁴⁷ it may be supposed that *Toxoplasma* can pass to the foetus without necessarily incurring infection of the placenta.

No attempts were made to treat toxoplasmosis with medicaments. The information on the results of such treatment vary widely.^{53, 55} In experimental toxoplasmosis on the mouse and rabbit it was possible to prolong the lifetime by treatment with sulphathiazole and sulphapyridine. In man similar treatment has not yielded such convincing results, but satisfactory progress has been reported.⁵⁵ The cases in Table 33 received no medicaments, so that one cannot ascribe to sulpha therapy the fact that the child did not contract congenital toxoplasmosis, though the mother presented high dye test titres. The good results reported of the use of sulphonamides and pyrimethamine in various forms of toxoplasmosis may be regarded as promising, but not infrequently the results have been judged too uncritically.

CHAPTER VIII

TRANSFER OF ANTIBODIES ACROSS THE PLACENTA

SEROLOGIC STUDY

Antistreptolysin titre

In 50 cases the antistreptolysin titre for the foetal blood in the 3rd to 6th months was very low, with a mean of 32 units per ml serum. In the 7th and 8th months this had risen to 110 units, and at full term the mean for the cord blood was 159 units (Fig. 9). Higher titres were recorded for the maternal than cord blood in the 3rd to 6th***, and 7th to 8th months, the difference, however, being not significant for the latter period.

At full term on the other hand the relationship was reversed.

Antistaphylolysin titre

In 50 cases the antistaphylolysin titre for the foetal blood in the 3rd to 6th months was low with a mean of 0.26 units per ml, which rose in the 7th and 8th months to 0.40; at term the AS_ta for the cord blood was 0.57. Higher titres were recorded for the maternal blood in the 3rd to 6th and the 6th and 8th months***. At term the value was approximately the same for maternal and cord blood (Fig. 10).

Bact. coli agglutination titre

The results of the *Bact. coli* agglutination titre tests on the maternal and cord blood are given in Table 36.

The maternal serum agglutinated the O antigen in 19 per cent at serum dilution 1:320, while cord blood gave agglutination only for 0.6 per cent at a dilution of 1:160, with no agglutination at higher dilutions. For H antigen maternal serum gave agglutination in 11 per cent at serum dilution 1:320, while cord blood gave agglutination only for 2 per cent at serum dilution 1:160.

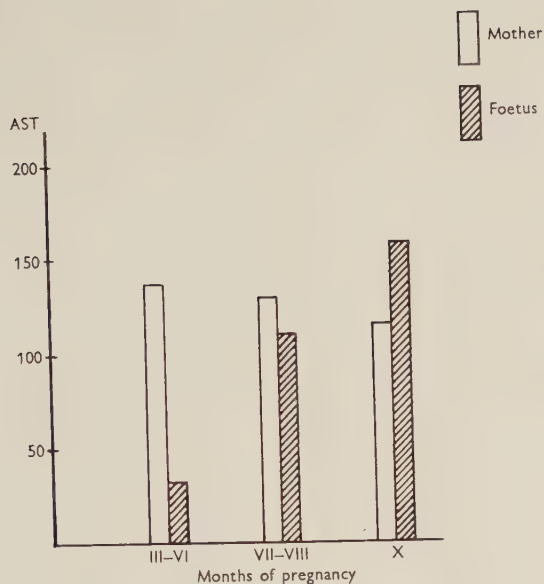


FIG. 9. Comparison of AST for mother and foetus at various periods of gestation.

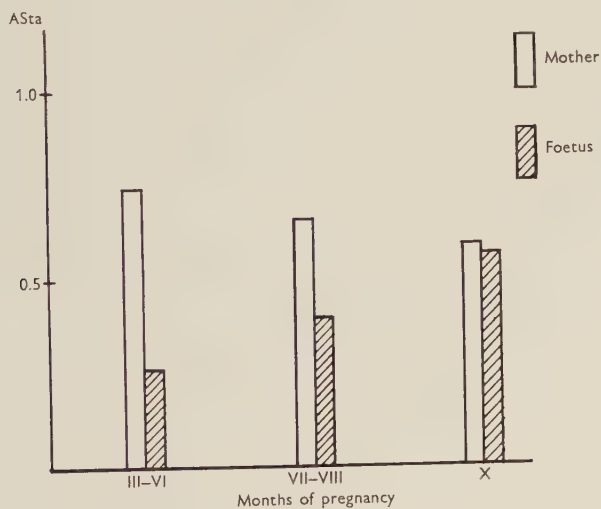


FIG. 10. Comparison of ASta for mother and foetus at various periods of gestation.

Table 37 shows the results of *Bact. coli* titrations in cases where the determination was carried out with an initial serum dilution of 1:20. For O antigen maternal serum gave agglutination at a dilution of 1:20 in 78 per cent of the cases while the corresponding number for cord serum was 3 per cent***. For

TABLE 36 *Bact. coli* agglutination titre for 147 mothers and newborn (cord blood)

	No agglutination 1:80		Agglutination							
			≥ 1:80		≥ 1:160		≥ 1:320		≥ 1:640	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Antigen O</i>										
Mothers	31	21	116	79	79	53	28	19	5	3
Newborn	142	97	5	3	1	0.6	0		0	
<i>Antigen H</i>										
Mothers	50	34	97	65	56	38	16	11	4	2
Newborn	129	87	18	12	4	2	0		0	

TABLE 37 *Bact. coli* agglutination titre for 88 mothers and newborn (cord blood)

	No agglutination 1:20		Agglutination											
			≥ 1:20		≥ 1:40		≥ 1:80		≥ 1:160		≥ 1:320		≥ 1:640	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Antigen O</i>														
Mothers	19	21	69	78	69	78	69	78	48	55	15	17	4	5
Newborn	83	94	3	3	2	2	2	2	1	1	0		0	
<i>Antigen H</i>														
Mothers	29	33	59	67	59	67	59	67	33	38	9	10	2	2
Newborn	65	73	15	17	14	16	13	15	4	5	0		0	

H antigen the difference between the serum from the mother and the cord was less marked but also highly significant.

Table 38 shows the results of *Bact. coli* agglutination for mothers and newborn in the 28 cases where the cord blood gave agglutination for O or H antigens in total dilution of 1:20. As the table shows, for O antigen there was agglutination in the cord blood at a serum dilution of 1:160 only in one case, whereas for H antigen 5 cases showed agglutination at this dilution.

Virus antibodies

In 72 cases of normal pregnancy the complement fixation test against *poliomyelitis* antigens 1, 2 and 3 was performed in maternal and cord blood. The series was unselected with regard to the possibility of polio in the district. The reaction was negative for type 1 in 78 per cent, for type 2 in 57 per cent

TABLE 38 *Bact. coli* agglutination titre for mother and newborn in which cord blood gave agglutination with O and/or H antigens at a total serum dilution of 1:20

Record no.	<i>Antigen O</i>		<i>Antigen H</i>	
	Mother	Newborn	Mother	Newborn
709	320	0	80	80
717	80	0	160	80
771	320	80	320	80
837	80	80	0	0
691	80	0	0	40
694	80	0	0	20
761	160	0	80	80
989	80	0	640	160
1020	160	20	640	0
1122	320	0	160	20
1191	320	0	80	80
1199	160	0	320	40
1217	80	0	160	20
1276	320	20	160	0
1298	0	0	0	80
1303	0	0	80	80
1362	160	0	160	20
1084	80	0	160	160
1399	160	0	160	160
1500	80	0	160	160
1515	0	0	160	80
1527	80	0	320	20
1531	160	0	320	40
1627	0	20	0	0
1673	640	160	320	0
2294	160	80	80	0
2306	160	0	160	80
2335	320	0	160	160

and for type 3 in 36 per cent of the cases. The corresponding figures for the cord blood were 72, 56 and 28 per cent. Where the CF test was positive the titre values were approximately the same in maternal and cord blood (Table 39).

In 30 unselected cases of normal pregnancy the complement fixation test for *influenza* A and B antigens revealed closely similar titres in the maternal and cord blood, with a slight tendency to higher values in the latter (Table 40).

In 55 cases of normal pregnancy the complement fixation test was performed for *parotitis* in maternal and cord blood. A paired comparison revealed a significant difference (Table 41).

TABLE 39 Complement fixation against antigens of polio types 1-3 for maternal and cord blood; normal delivery

Titre	Maternal blood			Cord blood		
	1	2	3	1	2	3
Negative	56	41	26	52	40	20
Positive						
1:2	11	20	17	11	15	15
1:4	4	10	18	8	17	23
1:8	1	1	11	1	—	13
1:16	—	—	—	—	—	1
Total cases	72	72	72	72	72	72

TABLE 40 Complement fixation against A and B antigens of influenza for maternal and cord blood; normal delivery

Titre	Maternal blood		Cord blood	
	A	B	A	B
≤ 1:5	17	24	15	18
1:10	4	3	4	8
1:15	4	—	3	1
1:20	3	1	3	1
1:30	2	1	2	1
1:40	—	—	2	1
1:60	—	1	1	—
Total cases	30	30	30	30

TABLE 41 Complement fixation against parotitis for maternal and cord blood; normal delivery

	No.	Mean ± S.E.	S.D.	<i>t</i>
Maternal blood	55	1.37 ± 0.05	0.38	2.0*
Cord blood	55	1.44 ± 0.05	0.36	

Dye and complement fixation test

The response to dye and complement fixation test was found to be the same for maternal and cord blood at term (Table 42).

TABLE 42 Comparison of dye test and complement fixation test titres for maternal (T_M) and cord (T_N) blood; normal delivery

Dye test	No.	%	Complement fixation test	No.	%
$T_M = T_N$	504	81.4	$T_M = T_N$	226	83.4
$T_M > T_N$	31	5.2	$T_M > T_N$	19	7.0
$T_M < T_N$	64	10.7	$T_M < T_N$	26	9.6
Total	599		Total	271	

BIOCHEMICAL STUDY

Electrophoretic examination of sera

A comparison was made between the amounts of plasma proteins for the mother and the foetus for various stages of pregnancy (Table 43). The first experiments showed that the relative content of γ -globulin of foetal sera is very low during the early months but that it rises towards term. It was further observed in the majority of sera from 4–5 month foetus, and occasionally in the serum from older foetus, that there was a previously undescribed component with a remarkably high velocity of migration of about $16 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1}$ at pH 7.6. (Fig. 11).

Table 44 presents the results of some 10 electrophoresis experiments on sera from foetus of various ages. The γ -globulin displays rather large variations. The studies are too few for statistical analysis, but it is seen from Fig. 12 & 13 that the relative content of γ -globulin increases slowly until the end of the foetal

TABLE 43 Plasma protein components in maternal and foetal blood

The figures are percentages

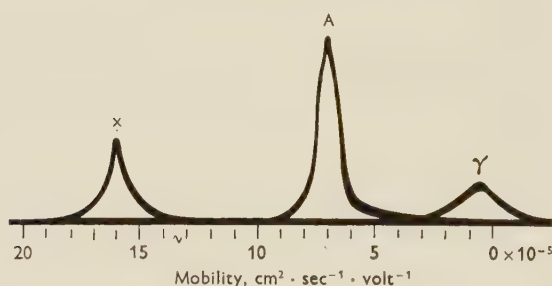
Case no.	Months pregnant	Maternal plasma					Foetal plasma					New component
		A	α	β	γ	F	A	α	β	γ	F	
463	X	56	11	13	16	4	56	9	4	24	1	—
2086	IV	49	12	12	22	5	56	7	8	0	—	28
2090	V	52	14	9	22	3						
708	VIII	49	20	11	18	2	54	6	7	19	4	10

A = albumin; F = fibrinogen; α -, β -, γ -globulin.

TABLE 44 Electrophoretic analysis of foetal serum proteins

The figures are percentages

Case no.	Months pregnant	Ascending boundary					Descending boundary				
		A	α_1	α_2	β	γ	A	α_1	α_2	β	γ
12320	IV	59.5	7.0	8.9	18.3	6.3	72.5	4.8	8.6	8.8	5.3
6441	V	57.8	4.4	10.5	13.5	4.1	53.8	5.3	12.2	10.6	7.2
6623	V						68.9	5.4	4.6	6.9	1.0
6496	V						62.7	6.2	8.4	9.1	4.2
1520	VI	71.8	4.4	6.9	9.0	7.9	77.3	4.8	6.1	8.2	3.6
6573	VI	71.9	5.9	4.1	8.2	0.5	62.4	9.3	6.9	8.7	0.6
7305	VI	73.4	3.4	5.5	10.4	2.8	73.7	4.3	5.4	9.7	1.7
4062	VII	63.7	6.1	9.8	9.6	10.8	66.1	6.6	9.9	7.5	10.1
1282	VIII	58.6	8.9	12.3	13.6	6.7	66.7	7.0	12.9	8.9	4.5
1280	IX	61.2	5.0	7.7	9.7	16.5	65.0	5.6	8.1	9.4	11.9
1270	X	58.8	5.0	9.4	8.2	18.6	57.3	7.0	9.1	7.8	18.8
6882											
Amniotic fluid	IV	76.3	9.2	—	8.1	6.4	73.6	9.6	—	8.1	8.7

A = albumin; α -, β -, γ -globulin.FIG. 11. Electrophoretic pattern of foetal serum showing albumin (A), γ -globulin and a new component (x); foetal age 4 months.

development, when the increase accelerates. The increase then, expressed as the absolute content of total protein, shows a similar course; the absolute content of γ -globulin will display a still greater increase towards term.

As regards the other components, no statistical analysis was performed, but the variations were rather great so that a large series would be required to obtain a reliable result. It would appear, however, that the relative amount of α_1 - and α_2 -globulin is rather constant during the whole development of the foetus, whereas the relative amount of β -globulin showed a tendency to fall.

The relative amount of albumin appears to reach a maximum in the 7th month.

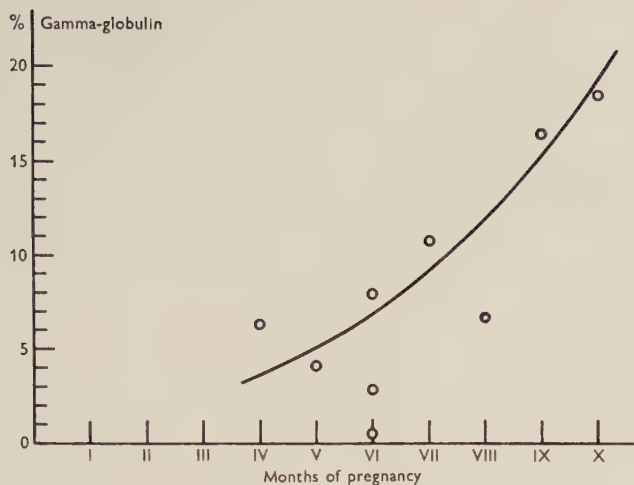


FIG. 12. Foetal γ -globulin level from 4th month of pregnancy to term.

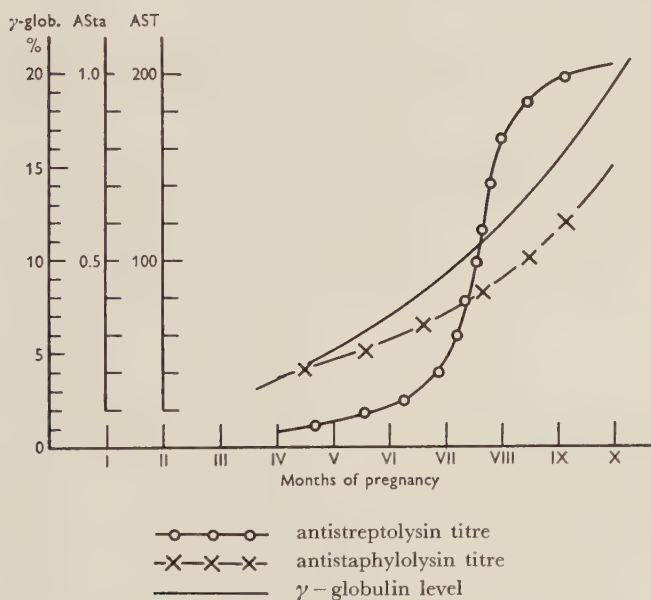


FIG. 13. Foetal γ -globulin level, antistreptolysin and antistaphylolysin titres from 4th month of pregnancy to term.

Electrophoretic study of maternal serum

Electrophoretic analyses were performed on pooled sera from gravidae at 3rd to 4th, 6th to 7th, and 10th months and on retroplacental serum. The results are shown in Fig. 45 and Table 45.

Table 45 shows that the amount of β -globulin increases throughout pregnancy and that there is a corresponding decrease in the albumin. There seems to be no marked change in the other globulins except a possible rise in the α_2 -globulin at term.

Table 45 Electrophoretic analysis of serum proteins in normal pregnancy

The figures are percentages

	Months of pregnancy			Retroplacental serum
	3rd	6th and 7th	10th	
Albumin	51.5	47.4	42.7	43.3
α_1 -globulin	8.5	10.4	9.4	8.4
α_2 -globulin	12.4	13.4	17.1	14.3
β -globulin	16.0	17.7	19.3	19.5
γ -globulin	11.8	11.1	11.5	14.5

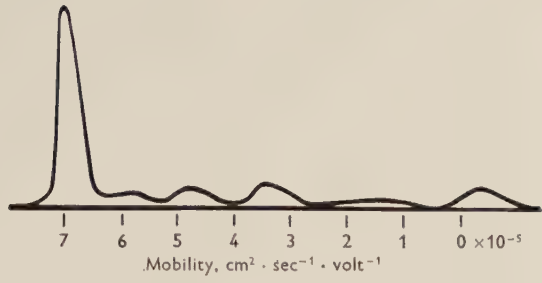
Ultracentrifugal examination of foetal serum

Figure 15 shows an ultracentrifuge diagram of the serum from a foetus in the 5th month after centrifuging for 80 minutes in a field of 100,000 g . A distinct peak with sedimentation constant $s = 4.5$ approx. is seen in the figure. This peak may be identified as albumin. There is also a smaller peak with a sedimentation constant of about 6.8. Although this peak is less distinct, it is probably to be attributed to γ -globulin. The α -globulins have already disappeared from the field of observation of centrifuged cells under these conditions, whereas the sedimentation constant for β -globulin is much the same as for albumin, so that it is impossible to distinguish between the two. The diagram gives 0.50 Gm/100 ml for albumin and 0.07 Gm/100 ml for γ -globulin, approximately.

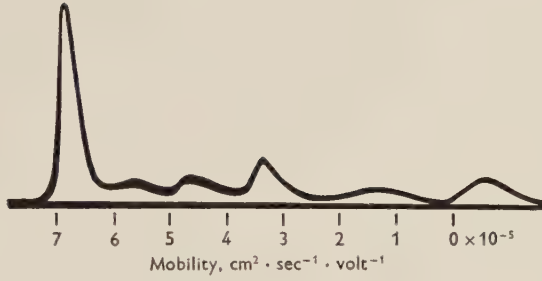
Fractionation

Fractionation according to Cohn's method 6 was performed on some 20 sera from maternal and cord blood at normal delivery at full term. The fractionation was performed as described in Chap. III, p. 31. The results are

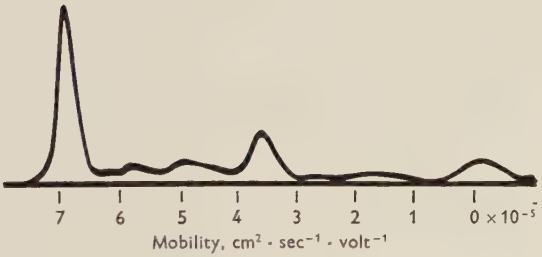
14 a. Maternal serum 3-4 months.



14 b. Maternal serum 6-7 months.



14 c. Maternal serum at term.



14 d. Retroplacental serum.

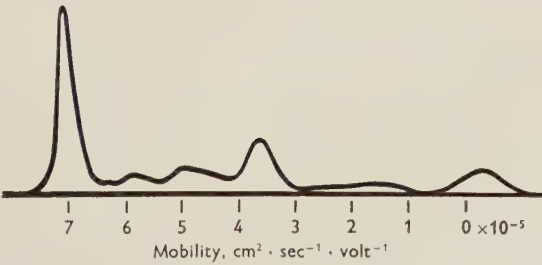


FIG. 14. Electrophoretic analysis of maternal serum at various stages of pregnancy.

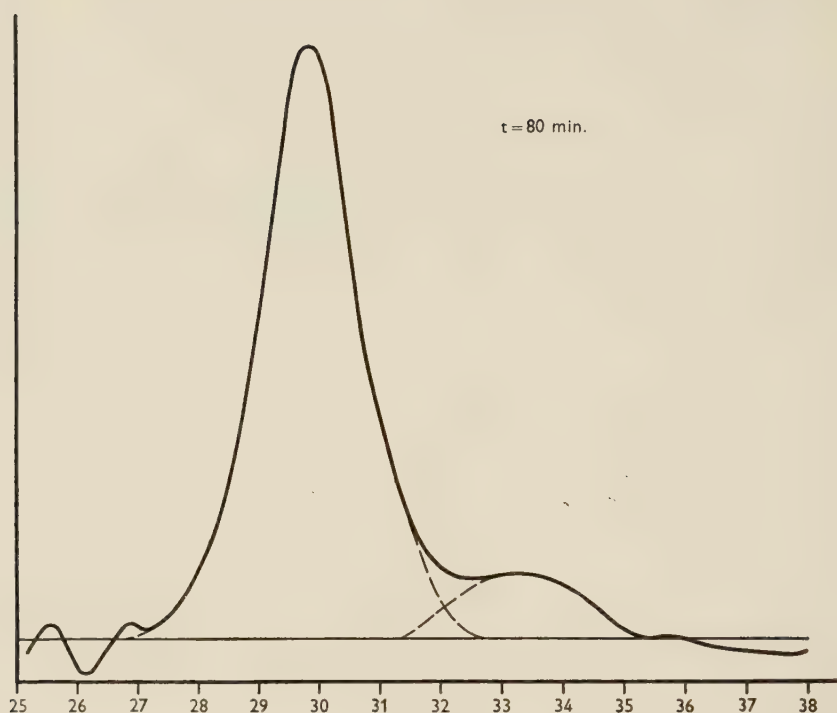


FIG. 15. Ultracentrifugal examination of foetal serum.

given in Table 46. Protein values were calculated from $6.25 \times$ nitrogen and nitrogen was determined according to Kjeldahl. As is seen from the table the relative albumin content is higher in the cord than in the maternal blood. In order to obtain the pure γ -globulin, sub-fractionation of Cohn's fraction II +

TABLE 46 Results of fractionation of maternal and umbilical cord serum (Cohn's method 6)

	Maternal blood		Cord blood		Principal fraction
	Protein ^a	% total protein	Protein ^a	% total protein	
Whole serum	6.43		5.92		
Fraction I	0.29	4.5	0.17	2.8	cold insoluble globulins
Fraction II + III	2.26	35.2	1.83	30.8	$\beta + \gamma$ -globulin
Fraction IV: 1	0.87	13.6	0.42	7.2	α -globulin
Fraction IV: 4	1.40	21.7	0.22	3.6	$\alpha + \beta$ -globulin
Fraction V	1.54	23.9	2.86	48.2	albumin
Final supernatant (incl. nonprotein N)	0.07	1.1	0.42	7.4	

^a Gm per 100 ml serum.

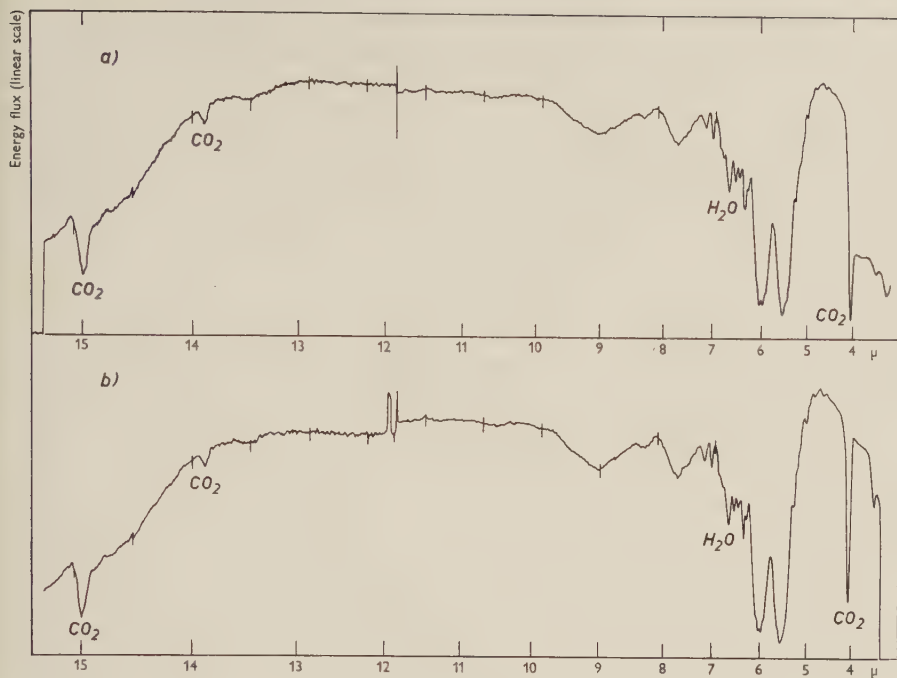


FIG. 16. Infrared spectroscopy of foetal and maternal γ -globulin at term. (a) foetal γ -globulin (KBr disc containing 0.63 mg γ -globulin) (b) maternal γ -globulin (KBr disc containing 0.65 mg γ -globulin).

III was performed in the manner described by Nietschman *et al.* (see Chap. III, p. 31), and the results are given in Table 47.

As is seen from the tables the relative content of γ -globulin is greater in the cord blood than in the maternal blood whereas the β -globulin content is greater in the mother, which might have been expected on account of the electrophoretic analyses.

Infrared spectroscopy

Fig. 16 shows a comparison of the infrared spectra of foetal (a) and maternal (b) γ -globulins. The curves give directly the energy flux on the detector. The vertical marks are reference marks, and in the centre of the two curves there is a discontinuity caused when one of the mirrors was changed. The γ -globulin absorption is seen between 5 μ and 11 μ beside the bands of atmospheric carbon dioxide and water. The spectra of foetal and maternal γ -globulin are identical, which bears out the view that the foetal γ -globulin is transferred from the mother.

TABLE 47 Subfractionation of fractions II and III (Nietschman's method)

Fraction ^a	Maternal serum		Cord serum		Fraction contains principally
	Protein ^b	% protein in II + III	Protein ^b	% protein in II + III	
III	1.76	77.8	1.04	57.1	β -(γ -)globulin
II	0.48	21.3	0.78	42.5	γ -globulin
Final supernatant (incl. nonprotein N)	0.02	0.9	0.01	0.4	
Total	2.26		1.83		

^a Corresponding approximately to Cohn's scheme.

^b Gm per 100 ml serum.

DISCUSSION

The results of antistreptolysin, antistaphylolysin, dye and complement fixation tests against polio, influenza and parotitis indicate that these antibodies occur to the same or higher extent in cord as in maternal serum. Production of antibodies has not been found to take place in placenta or in the foetus.⁴⁴ This seems to imply that they are transferred from mother to foetus and this mainly occurs during the later part of the pregnancy.^{135, 211}

The results with regard to *Bact. coli* agglutinins, which were only demonstrated in small amounts in cord blood, are in contrast to the other findings. Bearing in mind the possibility of non-specific agglutination, a positive reaction at a serum dilution of 1:320 was indicated as significant while agglutination in a serum dilution of 1:160 was designated as doubtfully positive. With these criteria, none of the 147 cord sera studied gave positive *Bact. coli* agglutination, while 30 per cent of the mothers reacted positively with O or/and H antigen. Earlier studies had suggested that *Bact. coli* agglutinins were able to pass the human placenta, but that there was a tendency for the agglutinins to be filtered off.

The fact that *Bact. coli* agglutinins are occasionally found in cord blood may possibly be ascribed to defects in the placenta, which might cause abnormal permeability. Such defects have been found in the human placenta on histologic examination.¹⁴⁷

The reason for the difference between antistreptolysin and antistaphylolysin on the one hand, and *Bact. coli* agglutinins on the other, as regards their occurrence in cord blood is not clear. There may possibly be some difference in the size or shape of the molecules in the respective antibodies. The possible significance of these various modes of occurrence of antibodies in cord blood

from the standpoint of immunity to the infection in question, constitutes an important problem which still requires further study. However, some interesting points may be mentioned in this connection. It has long been known among veterinarians that calves that do not receive colostrum often die from some form of diarrhoea. In a classic study¹⁸⁷ in this field coliform bacteria were found in the spleen, liver, and kidneys of calves that had died from this cause, and it was established experimentally that colostrum had a protective effect. It was stated that the placenta of the cow was impermeable to coli agglutinins since these are lacking in the newborn calf despite the fact that the dam may have a high titre. This has later been confirmed in the case of the cow, horse, sheep and pig. (See also Table 3.) In man the antibody content of colostrum is also considerable, but whether the newborn child can utilize these antibodies is doubtful.^{151, 152}

Electrophoretic studies of maternal and foetal sera have been performed by several workers since the middle of the nineteen forties, when the electrophoresis technique was developed. Electrophoretic analyses of *maternal* sera throughout the pregnancy have shown an increase in the α - and β -globulins and a decrease in albumin with the Tiselius' apparatus and the moving boundary technique.^{120, 142}

Later on many investigators have studied maternal sera at various stages of pregnancy using zone electrophoresis, especially paper. The decrease in albumin and increase of α - and β -globulin were confirmed, and some workers have suggested a decrease in γ -globulin during the course of pregnancy (see Table 4). This was not confirmed in this study; however, there was an increase in the γ -globulin content of retroplacental serum. A decrease in albumin and an increase in α_2 - and β -globulins were established. This was also confirmed by the fractionation experiments performed according to Cohn and Nietschman.

Several studies^{9, 69, 142} have shown that there is an increase in albumin and γ -globulin and only small changes in α - and β -globulins during the foetal development.

In a recent study²² by paper electrophoresis a component with a velocity of migration between that of albumin and α_1 -globulin was found in foetal sera in the first half of pregnancy. The amount of the component was as high as 10 per cent of the total protein. In this and in earlier studies¹³⁵ a similar component was found in foetal sera from the 4th and 5th months of pregnancy. However, the velocity of this component was much higher than that of albumin and therefore it is possible that the two components are not identical.

CHAPTER IX

SEROLOGIC AND ESR STUDY OF PATHOLOGIC CASES

ABORTION

The incidence of abortion is difficult to establish and it is likewise no simple matter to determine how many of the abortions are spontaneous. From a review of the literature Wetterdal²²³ concluded: "It must be accepted that a large body of strong evidence indicates that at least 10 per cent of all pregnancies are terminated by spontaneous abortion." It seems to be an established fact that various infectious diseases may give rise to injury to the foetus and abortion. Among the patients of this series that were studied from the beginning there were so few cases of abortion that comparison with the normal group would have been worthless. Supplementary serologic examinations were therefore performed on a non-selected series of abortion—both febrile and afebrile. In this supplementary series blood tests were taken for AST, ASta, DT, CF and ESR. It proved impossible to establish which of the abortions were spontaneous. The series was therefore divided into abortions with and without fever; both groups then definitely included cases of criminal abortion, although they were certainly more plentiful in the febrile group.

Antistreptolysin titre

The percentage number of patients having an AST above 200 units per ml was greater in the abortion group than in the normal group; age was controlled (Table 48). A similar comparison between febrile and afebrile abortions showed a higher percentage in the febrile group (Table 49). The infections in abortion are to some extent due to haemolytic streptococci, which were found in cultures in various cases.

Antistaphylolysin titre

A comparison in respect of ASta above 2.0 units per ml between the abortion group and the normal gravidae in the corresponding period of pregnancy revealed no significant difference for any age group. A similar comparison between abortion cases with and without fever revealed no significant differences (Table 48).

TABLE 48 Positive response to AST > 200 and AS_ta > 2.0 units per ml serum in 2nd and 3rd months of normal pregnancy and early abortion

Age (years)	Normal pregnancy			Abortion			Differ- ence
	Total	No.	%	Total	No.	%	
<i>AST > 200</i>							<i>t</i>
≤ 24	100	31	31.0	58	32	55.2	3.8***
25-29	74	21	28.4	63	25	39.7	
30-34	61	9	14.8	40	12	30.0	
≥ 35	39	7	17.9	64	19	29.7	
<i>AS_ta > 2.0</i>							%
≤ 24	112	6	5.4	58	5	8.6	3.2°
25-34	127	5	3.9	99	3	3.0	0.9°
≥ 35	40	1	2.5	68	2	2.9	0.4°

TABLE 49 Positive response to AST > 200 and AS_ta > 2.0 units per ml serum in afebrile and febrile early abortion

Age (years)	Afebrile			Febrile			Differ- ence
	Total	No.	%	Total	No.	%	
<i>AST > 200</i>							<i>t</i>
≤ 24	42	21	50.0	16	11	68.8	4.0***
25-29	48	15	31.3	15	9	60.0	
30-34	28	5	17.9	12	7	58.3	
≥ 35	55	13	23.6	9	6	66.7	
<i>AS_ta > 2.0</i>							%
≤ 24	42	2	4.8	16	3	18.8	14.0°
> 24	131	3	2.3	36	2	5.6	3.3°
Total	173	5	2.9	52	5	9.6	6.7*

Dye test and complement fixation test

In a comparison between the frequency of a positive response to the dye test ($\geq 1:50$) in the abortion group and in the normal series, age being controlled, a significantly higher frequency was found in the former (Table 50). A similar comparison between febrile and afebrile abortions revealed a slightly higher frequency in the latter group. Corresponding comparisons between the frequency of positive response to complement fixation test showed no significant differences.

TABLE 50 Positive response to dye test in normal and abortive pregnancy, according to age

Maternal age	Total no.	DT $\geq$ 1:50 No.	%	Total no.	DT $\geq$ 1:50 No.	%	<i>t</i>
<i>Normal pregnancy</i>				<i>Abortion</i>			
≤ 24	207	34	16.4	30	14	46.7	3.52***
25-29	153	34	22.2	37	14	37.8	
30-34	114	26	22.8	28	10	35.7	
≥ 35	73	15	20.5	35	8	22.9	
Total	547	109	19.9	130	46	35.4	
<i>Afebrile abortion</i>				<i>Febrile abortion</i>			
≤ 24	15	7	46.7	15	7	46.7	2.13*
25-29	21	11	52.4	16	3	18.8	
≥ 30	41	14	34.2	22	4	18.2	
Total	77	32	41.6	53	14	26.4	

Erythrocyte sedimentation rate

Earlier studies¹³⁷ showed an elevated ESR in cases of abortion and threatening abortion. In a recent study¹⁴⁹ it was found that the ESR was about the same in spontaneous abortions as in normal pregnancy at corresponding period of gestation. In criminal abortion, however, it was found that ESR was rather often increased. In previous studies¹³⁷ an account has been given of the ESR in normal and pathologic pregnancy. As the normal curve for the ESR was obtained from the normal group it will be quoted here (Table 51). In the abortions the ESR was significantly higher than in normal pregnancy (Table 52).

STILL BIRTH

In this series there were 34 cases of still birth; in 20 the birth weight exceeded 2500 Gm. All cases were subjected to postmortem examination and the following causes of death were established: atelectasis and intra-uterine asphyxia 23 cases (2 cases of aspiration pneumonia), malformations 5, rupture of the tentorium 2, erythroblastosis one case, with 3 cases not determinable.

In one case of intra-uterine asphyxia histologic examination disclosed pronounced signs of aspiration and foreign material in the respiratory passages and the alveoli. Culture samples from lung tissue revealed *Bacteroides*. During pregnancy there was no clear difference in respect of the antistreptolysin and antistaphylolysin titres between the normal group and mothers of stillborn children, but in some cases there were strikingly high titres for the latter, of which the following are two examples.

TABLE 51 The erythrocyte sedimentation rate in normal pregnancy and postpartum

		No. of cases	ESR (mean)	Mean $\pm 2 \times \text{S.D.}$
<i>Months of pregnancy</i>	II	40	11	2-27
	III	660	16	4-28
	IV	545	23	5-40
	V	637	26	9-44
	VI	866	30	10-49
	VII	848	34	12-57
	VIII	832	39	15-63
	IX	1013	45	17-73
	X	1082	48	19-76
<i>Weeks postpartum</i>	1	444	54	20-105
	2	73	29	9-61
	3	202	18	4-42
	4	84	16	4-34
	5	73	12	3-27
	6	63	11	2-27
Nonpregnant control group		400	8	3-15

TABLE 52 Erythrocyte sedimentation rate in normal pregnancy and abortion

Months pregnant	Normal pregnancy		Abortion		Difference
	ESR (mean)	No. of cases	ESR (mean)	No. of cases	
II	11	40	28	41	17***
III	16	660	33	105	17***
IV	23	545	56	21	33***
V-VI	28	1503	64	23	36***

CASE: M. E. A., a 20-year-old single primigravida examined at the antenatal clinic for the first time in the 2nd month of pregnancy. She had previously been quite healthy. There were no subjective symptoms.

The size of the uterus corresponded to the stage of pregnancy. Height 160 cm, weight 54.5 kg. Vaginal examination revealed no pathologic signs. Heart and lungs were normal. Blood pressure 150/90, haemoglobin 63 per cent. WR negative, Rh negative, AST 2200, AS_ta 0.28, ESR 45 mm in one hour. No albumin in the urine.

The patient did not attend for examination until the 6th month; in the meantime she had had no trouble. On this occasion: weight 54 kg, blood pressure 140/100, no albumin in the urine, AST 1600, AS_ta 0.25, ESR 34 mm in one hour.

When she attended for examination in the 7th month the blood pressure had risen

to 170/115, weight 57.2 kg. There was albumin in the urine, and oedema. AST 800, ASta 0.32. Ophthalmoscopy showed fundus hypertonicus II. The electro-encephalogram showed a severe pathologic activity in the anterior part of the cortex cerebri.

She gave birth to a dead child, a boy, with a weight of 970 Gm.

Biopsy revealed numerous subserous haemorrhages in the pleurae and pericardium.

Diagnosis: asphyxia intra-uterina.

CASE: D. G. B., a 27-year-old married primigravida. As a child this patient had had morbilli, parotitis and scarlatina. She had her last menstruation on 28th February and presented typical rubella on 31st March. The pregnancy afterwards ran a normal course until 2 days before delivery, when the foetal movements ceased. There was spontaneous labour, with vertex presentation of a stillborn boy weighing 2400 Gm. There was no winding of the cord round the trunk.

On biopsy the heart was found to be normal in size and shape. The arteria pulmonalis passed from the right chamber and was slightly wider than normal. The ductus Botalli was wide and the wall had a slightly wrinkled appearance. At the boundary of the arcus and the descendens the aorta was stenosed and the lumen was no wider than a match. The heart muscle contained numerous areas of haemorrhage similar to those over the thymus and on the pleural membrane. The lungs were collapsed and dark reddish blue in colour. The abdominal organs presented no remarkable changes. The postmortem diagnosis was aortic stenosis.

The mother has since given birth to a normal child. The WR was negative and the Rh positive. The serologic tests during pregnancy are summarized thus:

	Months pregnant						Weeks postpartum	
	III	IV	VI	VIII	IX	X	1	3
AST	400	200	200	100	400	200	200	200
ASta	1.8	2.0	1.0	1.5	—	1.4	1.4	1.4
ESR	5	32	27	25	43	38		16

The days just before delivery she had 11 pro mille albumin in the urine, but this value fell after delivery; 6 weeks later, however, it was still 0.5 pro mille. The systolic blood pressure, which prior to delivery was about 160–170, fell to 120–130. The diastolic pressure did not fall to lower than 90 during the period of observation.

One month after delivery the AST was still 800 and the ASta 0.7 units. On the occasions of three subsequent deliveries the urine albumin was high and there was hypertonia. Eye fundi examinations revealed fundus hypertonicus II. Heart and lung radiographs were noncontributory. Creatine clearance at repeated checks showed an F value of 100. The patient probably had a chronic impairment of the kidneys.

TOXAEMIA OF PREGNANCY

A fairly common complication of pregnancy is toxæmia. The incidence was roughly the same in this case series as is generally reported in the literature—that is, between 6 and 10 per cent. For a diagnosis of toxæmia the patient

TABLE 53 AST and ASa in normal pregnancy and toxæmia of pregnancy, tenth month

Titre groups (units per ml serum)	Normal pregnancy				Toxæmia of pregnancy			
	< 30 years		≥ 30 years		< 30 years		≥ 30 years	
	No.	%	No.	%	No.	%	No.	%
<i>Antistreptolysin titre</i>								
25-50	103	14.7	77	20.3	16	15.0	15	30.0
51-100	209	29.8	131	34.5	32	29.9	20	40.0
101-200	241	34.3	121	31.7	31	29.0	6	12.0
201-400	123	17.5	47	12.4	21	19.6	3	6.0
401-800	25	3.7	4	1.1	5	4.7	4	8.0
> 800	1	0.0014	—		2	1.8	2	4.0
Total	702		380		107		50	
<i>Antistaphylolysin titre</i>								
≤ 0.25	96	13.7	70	18.4	11	10.4	6	12.2
0.28-0.5	238	34.0	146	38.4	30	28.3	20	40.8
0.56-1.0	259	37.0	118	31.1	46	43.4	19	38.8
1.1-2.0	93	13.3	43	11.3	15	14.1	2	4.1
> 2.2	14	2.0	3	0.8	4	3.8	2	4.1
Total	700		380		106		49	

TABLE 54 Erythrocyte sedimentation rate in toxæmia of pregnancy

Months pregnant	Normal pregnancy		Toxæmia of pregnancy		Difference (mm/one hour)
	ESR (mean)	No. of cases	ESR (mean)	No. of cases	
V	26	637	33	47	7***
VI	30	866	40	70	10***
VII	34	848	47	96	12***
VIII	39	632	55	99	16***
IX	45	1013	61	121	16***
X	48	1082	65	124	17***

was required to have at least two of the symptoms: hypertension, oedema and proteinuria. There were no cases of eclampsia in this series.

The cause of this disease is still unknown but there is no reason to believe that haemolytic streptococci and staphylococci play an aetiological role. It has long been supposed, however, that women who have had scarlatina more often contract toxæmia when they become pregnant. To ascertain whether there was any rise in the AST and ASa in toxæmia a comparison was made

between the titres in this disease and in the normal group (Table 53). The incidence of cases was about the same in various titre groups, except that cases of toxæmia over 30 years of age more often had an AST of more than 400 units. This rise was significant***. The reason for this was probably that in this age group of toxæmia there were several cases of pre-eclampsia superimposed on chronic renal disease. There were more cases in the toxæmia group with an AS_ta titre of over 2.2 units but the difference was not significant.

With respect to *Bact. coli* agglutinins there was no significant difference between the toxæmia and the normal group.

Table 54 shows a comparison of the ESR for 125 subjects with toxæmia, and the corresponding group of normal pregnancies. The rate was consistently higher in cases of toxæmia, the differences being significant. Of particular interest and practical importance is the occurrence of a higher ESR as early as the 5th and 6th months in cases of toxæmia, although clinical signs of the condition did not appear until later.

TOXOPLASMOSIS

As an example of lymphadenopathy during pregnancy where the child was born with signs of congenital toxoplasmosis the following case may be given.

CASE. 204657. U. M. V.-I., a 26-year-old primigravida. The patient had always been healthy. The last normal menstruation was on 28th November, 1956. There were troublesome periods of vomiting during the first four months of pregnancy. At the beginning of March, 1957, the patient complained of pains in the right side of the neck. On examination revealed a painful enlargement of the lymphatics. Test excisions were taken for histologic examination which suggested toxoplasmosis. Serologic examination confirmed this suspicion. The patient presented no symptoms after some weeks and the pregnancy followed a normal course until one month prior to term, when pains and fluid discharge were experienced.

On 11th July, there was spontaneous birth in head presentation. The child, a boy, weighed 2900 Gm; length 50 cm. The head was strikingly large, with a circumference of 36 cm. The anterior fontanelle was 3 × 3 cm.

The placenta showed moderate deposits of calcium salts, but no infarcts.

Serologic examination.

Date	DT	CF
18.5 1957	1:1250	1:30
1.6	1:1250	1:120
11.7	1:1250	1:120
5.8	1:1250	1:120
4.9	1:1250	1:120
25.10	1:1250	1:120
19.12	1:1250	1:120
18.1 1958	1:1250	1:30

The child was transferred on the same day to the Sachs' Children's Hospital, where the following observations were reported. 11th July, moderate hydrocephalus, but status otherwise normal. Cranial radiography, 16th July, showed bilateral small semi-circular areas of intracranial calcification, located possibly at the site of the plexus chorioideus.

Eye examination (15th and 16th July): bilateral uveitis and retinochoroiditis of a toxoplasmotic nature. The process was considered to be progressive.

Cerebrospinal fluid. On 12th July, as on several subsequent occasions, the fluid was yellow, and gave marked positive albumin reactions. In the lumbar samples taken on 18th July *Toxoplasma* was demonstrated. On another occasion (3rd September) the sample was taken from a lateral ventricle of the brain, and *Toxoplasma* was also found then.

EEG (8th August) showed no pathologic signs for the age.

Treatment. Sulphonamide (Gantrisin) was given, 1-3 tablets 4 times a day during the period 16th July to 4th August.

Course. No abnormal symptoms for the first weeks, but the child then became so lethargic that it was necessary to feed by means of the stomach tube. There were occasional bouts of fever for some days, with no other signs of infection. From the age of 5 weeks the tonus increased and he is now lying in the opisthotonus position. There is slow nystagmus. Eye examinations have shown that the choroiditis has healed with permanent changes in the eyes.

At the beginning of April the child was still living.

Serologic examination

Date	DT	CF
11.7.1957	1:6350	1:120
26.7.	1:6250	1:30
16.9.	1:1250	1:7.5

As an example of toxoplasmosis during pregnancy possibly giving rise to intra-uterine death of the foetus the following case may be given.

CASE. 214057. J. M. S., a 22-year-old primigravida. Last period 17th December, 1956. First examination on 4th March, 1957. Complained of slight pain in the left side of the neck. There was no eruption. There was painful enlargement of the lymphatics on the left of the neck. Gynaecologic examination revealed normal pregnancy in the third month. WR negative. Blood group A, Rhesus-positive.

Further examinations on 14th May disclosed no subjective symptoms. There were enlarged and slightly sensitive lymphatics on the left of the neck.

The size of the uterus corresponded to the stage of pregnancy. Movements of the foetus had been felt for two weeks. Further examination on 18th June revealed no subjective symptoms. The uterus had reached the navel plane. Foetal heart tones normal.

At examination on 15th July the patient reported abdominal pains on and off for the past week. No foetal movements experienced for the previous 2 weeks.

No foetal heart tones were heard. Vaginal examination revealed that the cervical canal was wide enough for two fingers and the membranes were bulging into the cervix.

In the evening of the same day there were pains and on 16th July a macerated foetus was delivered in breech presentation. Birth weight 1200 Gm, length 37 cm. Head 27 cm. There was no haemorrhage or infarcts in the placenta. At autopsy no cause of death could be established. There were no visible malformations. Histologic examination revealed such marked autolytic changes in the parts examined that no evaluation could be made.

Toxoplasmosis examination

Date	DT	CF
15.7. 1957	1:1250	1:480
5.8.	1:1250	1:960
11.11.1958	1:1250	1:480
2.1.	1:1250	1:240

In April, 1958, there were no symptoms, but delay in new pregnancy was recommended until the titre values had fallen further.

GENERAL DISCUSSION

The significant decrease in the antistreptolysin, antistaphylolysin and *Bact. coli* agglutinin titres during the course of pregnancy may be the result of several co-operating factors (Chapters IV, V and VI). Studies of the plasma from different periods of pregnancy by means of zone electrophoresis have suggested that there is a reduction in the γ -globulin content during the last months (Table 4), but this could not be confirmed by the more accurate technique used in this study. The results of the serologic and biochemical examinations would thus seem to be contradictory. However, compared with the serologic methods electrophoresis and plasma fractionation are fairly rough techniques, and the results of the serologic examinations may therefore be regarded as being the more reliable.

It has been held that all the γ -globulin does not consist of specific antibodies, and some workers have regarded it only as a carrier system for antibodies.²⁸ This may be the explanation for the divergency between the results of the serologic and biochemical studies.

There may also be a systematic error that influences the results of the serologic studies, and in this respect the following factors may be relevant. The considerable inter-subject variations in the titre values may influence the results of examination of one group of gravaide at the beginning and another group at the end of pregnancy. This source of error was eliminated, however, by intra-subject comparison between the various periods of pregnancy. There may also be seasonal variations in the titre values as a result of variation in the incidence of streptococcal and staphylococcal infections. As the study has been running for some four years such variations would have been smoothed out, however. In one study^{22b} of these titres in a tuberculosis series an increase in the AST and a fall in AS_ta was reported over a period of 10 years. The changes occurring slowly over a decade, even if they could be confirmed, would not influence the changes occurring over the rather short period of pregnancy.

The laboratory personnel were unaware of the period of pregnancy to which a particular blood sample belonged, so that no influence on the titre readings in this respect was possible. The error of the method associated with the performance of the tests is probably also of no significance. Duplicate tests for both AST and AS_ta displayed close agreement (p. 34). The error in the case of the *Bact. coli* agglutinin reaction was perhaps larger. However, a

larger error of the method would result in any existing difference being concealed, and there was a significant difference between the titre values at the beginning and end of pregnancy with respect to this reaction.

Among the other defence mechanisms of the body is phagocytosis, which constitutes a fairly important factor. Recent studies⁹⁴ indicate that there is a hormonal influence on phagocytosis, the process being inhibited by supply of cortisone. Whether or not phagocytosis changes during the course of pregnancy does not seem to have been examined.

During recent years considerable attention has been given to the properdin system and it has been regarded as being of importance in the natural immunity²⁸. Properdin is a protein with a molecular weight of about one million and constitutes about 0.03 per cent of the total protein. In contrast to the antibodies, properdin has a non-specific action. In electrophoresis properdin displays a velocity of migration similar to that of β -lipoproteins. The properdin content is stated to change with the seasons, with a higher value during the winter. There is no correlation with the γ -globulin content and cases of agammaglobulinaemia have presented a normal properdin level. How the properdin content varies during pregnancy, in mother and foetus, has apparently not been studied. In the presence of complement and magnesium ions properdin exerts a bactericidal action on gram negative bacteria, and neutralizes certain viruses and lyses red cells. It has been suggested that properdin is an essential factor in the dye test.^{61, 85}

In contrast to the AST, AS_ta and *Bact. coli* agglutinins, the antibodies against toxoplasmosis showed no fall in the titre values during the course of pregnancy. It is generally agreed that the dye test titres remain longer and only after several months do they show a tendency to fall, as is illustrated by the clinical examples reported in Chapter IX. Complement fixation test titres, which generally do not remain so long, might, on the other hand, be expected to fall, however. That this was not the case may be ascribable to the fact that several patients were probably suffering from sub-clinical toxoplasmosis during pregnancy, which involves an increase in the titre values that more than compensates any fall in the values due to the pregnancy itself. As toxoplasmosis during pregnancy may have deleterious effects on the foetus, it is important to attempt a diagnosis as early as possible. In the study of gravidæ routine examinations of any enlargement of the lymph nodes, especially in the neck, should be performed, and where there is unexplainable pain in the region of the neck the possibility of toxoplasmosis should be borne in mind. Serologic examination of toxoplasmosis during pregnancy should be performed to a greater extent than has hitherto been the case. If a diagnosis of toxoplasmosis is placed during the first trimester the question of interrupting the pregnancy arises. Acute toxoplasmosis during the first trimester will not infrequently result in death of the foetus and spontaneous abortion.²⁰⁰ During

the second and last trimesters there is a greater risk that infection of the foetus will occur, with congenital toxoplasmosis as a result. The diagnosis in such cases will often be made so late that under the law at present in force in Sweden legal abortion is impossible (as in the case described above, p. 86). Several of the cases of toxoplasmosis in the mother did not result in infection of the foetus. How great the risk of this happening is has not been definitely calculated. The uncertainty is illustrated by the case of U. K., sister-in-law of the patient U. M. When U. M. had given birth to a child with severe congenital toxoplasmosis her sister-in-law U. K. was in the 3rd month of pregnancy. Toxoplasmosis tests showed a dye test titre of 250 and this rose during the course of one month to 1250. In view of the risk of another case of a congenital toxoplasmosis in the same family permission was sought to interrupt the pregnancy, and this was granted by the Medical Board. However, when this permission was made known to U. K., she enquired as to the risk that her child would be infected by toxoplasmosis. As no answer to this question could be given, she decided to allow the pregnancy to take its normal course and she gave birth to a healthy child at full term. Eye examination showed no signs of toxoplasmosis and cranial radiographs revealed no calcification. During one month since the delivery the child has developed normally and presented no signs of hydrocephalus, at the beginning of April. Although this case series is too small to permit a definite conclusion to be drawn, it seemed that legal abortion on indication of toxoplasmosis in the mother was unjustified. This opinion has been voiced by earlier workers.²⁰⁰

In cases of still birth and abortion of obscure genesis, serologic examination for toxoplasmosis should be performed as a routine procedure. Some examinations performed earlier by methods that were not as reliable as the dye test may have given an inaccurate picture of the incidence with which toxoplasmosis gives rise to impairment of the foetus.

The placenta was earlier regarded as a semipermeable membrane across which various substances were transferred with varying facility, depending on the molecular size. Gases and substances of low molecular weight, generally less than 1000, are considered to be capable of passing the placental barrier by diffusion.⁶³⁻⁶⁴ Examples of these are sodium chloride, water, urea, and uric acid, and of these the concentration is approximately the same in mother and foetus. Other substances, such as calcium, inorganic phosphorus, ascorbic acid, amino-acids and nucleic acid occur in higher concentrations in the foetus and it is to day generally assumed that they are transferred by activity of the placenta.^{64, 140} As regards the transfer of glucose, diffusion has been thought an inadequate explanation, and some enzymatic carrier mechanism has been postulated.⁶⁴ Dextrane, which contains various fractions with molecular weights varying from 36,000 to 156,000 has been found to pass the placental barrier.²⁷

In the case of transport from the mother to the foetus of substances of im-

munologic importance it would seem that the correctness of the earlier opinion is open to question, viz., that it is the number of layers between the two blood streams that is the factor determining whether antibodies may pass from the mother to the child through the placenta (Table 2). In studies with the electron microscope pictures suggesting the possibility of transport of large molecules through large pores and through droplet transfer by pinocytosis have been seen.⁶⁴ There is at present no agreement on the nature of the placental transport mechanism, but diffusion, carrier systems, leakage through large pores and perhaps also pinocytosis are probably of significance.¹⁴⁰ Many earlier workers^{179, 180} on antibodies in the mother and foetus appear, however, to be in agreement with Grosser's scheme. Whatever the mechanism may be, various antibodies pass from the mother to the foetus to a varying degree. In this study antistreptolysin, antistaphylolysin, and antibodies against toxoplasmosis, poliomyelitis, influenza and parotitis epidemica were found in roughly the same concentration in the maternal and cord blood at full term. On the other hand, *Bact. coli* agglutinins generally do not pass the placenta, especially in the case of O agglutinins. This is in accord with the findings in other studies in respect of AST,²¹¹ AStA,¹²⁴ and antibodies against toxoplasmosis²⁰⁰ and poliomyelitis.¹²

A decrease in albumin and an increase in α_2 - and β -globulins were established in this study, and similar results have been obtained earlier by other workers (Table 4). Among the characteristic changes in the blood in pregnancy is an increase in the fibrinogen and this component probably contributes to the increased erythrocyte sedimentation rate during pregnancy. It is now 40 years since Fåhræus⁷⁰ presented his observations on the erythrocyte sedimentation rate. The literature on the erythrocyte sedimentation rate in normal pregnancy has been summarized in Table 1. A survey of the literature revealed that the findings on the ESR during pregnancy are incomplete; in particular, reliable figures for the physiologic variations are lacking. On the normal group of this case series, in addition to the serologic examinations, the erythrocyte sedimentation rate was measured in order to find the normal curve and the physiologic variations during the various months of pregnancy. Results of this study have been published separately and they are only quoted here. In the case of toxæmia earlier workers¹³⁷ have found varying values of the ESR. This case series is probably the first to indicate a significantly higher ESR in toxæmia than in normal pregnancy. It seems to be of particular interest that significantly higher values were obtained in the toxæmia group before other signs of toxæmia, such as albuminuria, oedema and hypertension became evident.

CONCLUSIONS

(1) There was a significant decrease in some antibody titres during the course of pregnancy; this possibly indicates a lower resistance to infections in the later stages of pregnancy, and pregnant women should therefore be treated with consideration.

(2) Determinations of the antistreptolysin and antistaphylolysin titres may be of value in non-specific infections during pregnancy, and the titre values should be evaluated as in non-pregnant subjects.

(3) Blood samples for the dye test should be drawn in cases of still birth and abortion of obscure genesis. The dye test might well be used more frequently in pre-natal care, especially if there is any sign of lymphadenopathy. If toxoplasmosis in pregnancy is suspected sulphonamides, pyrimethamine and immune γ -globulin therapy should be tried.

(4) Determinations of the erythrocyte sedimentation rate should be used much more than hitherto in pregnancy, and here the normal values found in this investigation may then be a guide.

SUMMARY

(1) The variation in resistance to infection has been followed in a series of gravaide throughout pregnancy. In addition to the usual clinical examinations antistreptolysin, antistaphylolysin and *Bact. coli* agglutination reactions and the Sabin-Feldman dye test were performed on blood samples every lunar month. The transfer of antibodies from mother to foetus was studied by serologic and biochemical methods. The use of these serologic reactions and the erythrocyte sedimentation rate was studied in a number of pathologic conditions during pregnancy.

(2) The case series consisted of 1887 gravaide with no history of specific infections such as active tuberculosis, syphilis, haemorrhagic nephritis, and/or of non-specific infections during the months immediately preceding pregnancy. In the case of 538 subjects pregnancy was complicated by various morbid conditions; 232 patients discontinued attendance. The remaining 1117 cases constituted the *normal group*. To test the representativity of the normal group comparison was made with a *control group*, the subjects for which were drawn at random from some 40,000 gravaide at the clinic.

(3) The following serologic reactions were used: antistreptolysin, antistaphylolysin and *Bact. coli* agglutination reactions; dye and complement fixation tests for toxoplasmosis, complement fixation test for poliomyelitis, influenza and parotitis. The biochemical methods used were: electrophoresis with Tiselius-Svensson's apparatus, plasma fractionation according to Cohn, sub-fractionation according to Nietschman, and infrared spectroscopy.

(4) The relationship between the antistreptolysin titre and the maternal age, marital status and birth weight was studied by means of an analysis of variance. The younger subjects showed significantly higher titre values; this confirms the results of other workers. The titres for single subjects were higher than for married subjects; this may possibly be due to the inferior social conditions of single women. Primigravaide showed slightly higher titre values than multi-gravaide. A significant decrease in the mean AST throughout the pregnancy was found; this may be ascribed to a decrease in resistance to infection.

(5) The relationship between the antistaphylolysin titre and the maternal age, marital status and parity was tested by an analysis of variance. No significant differences were found in respect of birth weight, marital status and parity. The mean titre value was generally higher for the lower age groups,

though the difference was significant only for the last trimester. As in the case of the AST, there was a decrease in the mean AS_ta during the course of pregnancy.

(6) *Bact. coli* agglutination reaction. In respect of the O antigen there was no difference between the age groups. In the case of the H antigen, however, no agglutination was obtained in the case of 50 per cent of the members of the ≤ 24 year age group, and of 23.5 per cent of the ≥ 25 year group, the difference being significant. The *Bact. coli* titres showed a significant tendency to fall during the course of pregnancy.

(7) The number of positive responses to the dye and the complement fixation tests increased with age; this confirms the results of other workers. In the case of at least one per cent of the patients the dye and/or the complement fixation test changed from negative to positive during the course of pregnancy, but none of the children from these mothers exhibited any sign of congenital toxoplasmosis.

(8) In 50 examined cases the antistreptolysin and antistaphylolysin titres for the foetal blood were very low in the 3rd to 6th months; by the 7th month the titres had risen considerably, and at term the value for the cord blood was equal to, or higher than, that for the maternal blood. However, with regard to *Bact. coli* agglutinins, only small quantities of the antibodies were found in the cord blood at term. Complement fixation tests performed in maternal and cord blood with respect to poliomyelitis, influenza and parotitis, gave approximately the same titre values for the two bloods. The responses to the dye and complement fixation test were also approximately the same for the cord and maternal blood at term.

Electrophoretic analysis of maternal blood at various stages of pregnancy, using the Tiselius apparatus and the moving boundary technique, disclosed a decrease in albumin and an increase in α_2 - and β -globulins. This was confirmed by plasma fractionation experiments.

Electrophoretic analysis of pooled *foetal* sera from various stages of pregnancy showed an increase in albumin and γ -globulin and only small changes in the α - and β -globulins. In foetal sera from the 4th and 5th months a new component was found that had a velocity of migration that was higher than that of albumin.

Pure γ -globulin was prepared by fractionation of maternal and cord serum at term. Infrared spectra of maternal and foetal γ -globulin were identical, which finding seems to support the view that foetal γ -globulin is transferred from the mother.

(9) *Abortion*. A significantly higher percentage of patients with abortion had an antistreptolysin titre of more than 200 units per ml serum than that of gravidæ at the same stage of normal pregnancy. A similar comparison between the afebrile and febrile abortions revealed significantly higher per-

centages in the latter group. In the case of the antistaphylolysin reaction a slightly higher percentage of subjects with a titre of more than 2.0 units was found in the abortion group. A similar comparison in respect of afebrile and febrile abortions showed no significant difference. In the case of the dye test ($\geq 1:50$) a significantly higher percentage of positive responses was found in the abortion group. In a similar comparison between afebrile and febrile abortions it was found that the number of positive responses to the dye test was higher in the afebrile group. The erythrocyte sedimentation rate was consistently higher in the abortion group than for the corresponding month in the normal group, the difference being significant. In this connection a table of the ESR (mean and standard deviation) during the various months of pregnancy is given.

Still birth. In the material there were 34 cases of still birth with various causes of death, the most common being intra-uterine asphyxia. In general, the titre values in pregnancies terminating in still birth were not significantly higher than in normal labour. A case with high AST and another with high dye test titres are reported.

Toxoplasmosis. A case is reported in which lymphadenopathy developed during the second trimester; the child presented severe congenital toxoplasmosis.

Toxaemia of pregnancy. A comparison of the AST and AS_ta in normal pregnancy and in toxaemia of pregnancy revealed a significantly higher number of cases with an antistreptolysin reaction at a titre greater than 400 units in the toxaemia group. In the case of the antistaphylolysin reaction a slightly higher number of subjects with a titre greater than 2.2 units was found in the toxaemia group.

The erythrocyte sedimentation rate was consistently higher in cases of toxaemia of pregnancy, the difference being highly significant. Of practical importance is the occurrence of higher ESR as early as the 5th and 6th months in cases of toxaemia, although clinical signs of the disease did not appear until later.

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